AN EYELESS MUTANT IN *DROSOPHILA HYDEI*

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INTRODUCTION

A new eye structure that shows marked variations in size depending upon the environmental conditions to which the culture is subjected, appeared in my stock of *Drosophila hydei* (Sturtevant) in 1917. Under certain conditions of temperature and moisture the eyes of this mutant stock appear as mere specks or are entirely missing, while the eyes of the same mutant stock under other conditions are very large, in fact indistinguishable from those of the wild type. Between these two extreme types all gradations appear. The mutant has been called "variable."

Cultures of variable subjected to warm dry conditions produce speck-eyed forms in large number; in fact the cultures at times become "pure" for speck eye. When the temperature at which the cultures are kept is reduced, the percentage of flies with large eyes increases. If the culture bottles are well supplied with moist food and kept at a temperature slightly below that of the living room the stock may appear "pure" for the wild-

type eye. Cultures kept in a fluctuating laboratory temperature, with food added at irregular intervals, give rise to a wide range from speck to normal-eyed forms with intermediate types. This was the condition under which we were studying the character for a long time after its origin, and until the key to the situation was found, the results of our crosses were most puzzling. By controlling the environmental conditions it can be demonstrated that variable is a recessive Mendelian character which seems to segregate freely from its normal allelomorph. Interest is added to this case since variable is linked with scarlet, another mutant obtained in this stock (HYDE 1915).

Before it became evident that the type of eye shown by the mutant depended upon certain environmental conditions, the results with the different crosses defied interpretation. Attempts to purify the stock by selection of the speck-eyed forms failed; for, after the process had been continued for some time and the stock bottles contained only speck-eyed flies, the next generation would apparently revert to the normal-type eye or give a range of eye sizes. The key to the situation was suggested by a comparison of the records covering a long period of time. The records showed that during the summer the speck condition of the eye was in evidence, while during the winter it was not so likely to appear. The result was explicable on the assumption that temperature was a determining factor in the realization of the character.

CONCERNING *Drosophila repleta* AND ALLIED SPECIES

It has become evident that the term *Drosophila repleta* includes more than a single species. In the American Naturalist for March 1915 STURTEVANT reported a new sex-linked mutant for *Drosophila repleta*. In the same number of the Naturalist I reported scarlet, a new mutant eye color for this same species (HYDE 1915). It is now certain that the *repleta* with which STURTEVANT worked and mine are two different species. They resemble each other so closely that despite the fact that I had bred both forms for a long time the difference was not discovered until many attempts at crossing had failed. Failure to cross is admitted to be sufficient ground for separating different species. Closer study has shown that the two differ also in certain morphological characters. In STURTEVANT'S type the lower side of the thorax including the basal segments of the legs is distinctly smoky in color, while in my type these parts are yellowish with the merest hint in some of a smoky color. In STURTEVANT'S type the yellowish segmental bands on the upper part of the abdomen are intersected by the alternating dark bands as they extend to the sides of the abdomen,

resulting in a demarkation on the side of the animal of a row of light spots. In my type these spots are only faintly in evidence. I have found STURTEVANT'S type in New York and Baltimore, but not in Indiana. My type is very common about fruit stores and orchards in Indiana, but so far I have not found it in Baltimore. There is still another type of *Drosophila repleta* that differs from the two types under discussion. It is characterized by rather large light spots on the sides of the abdomen. I have taken it during two different summers in the open woods in Indiana. It is very hard to breed in captivity. There would seem then to be at least three or four different species of this fly passing under the name *Drosophila repleta*. I have found three light males, of STURTEVANT'S mutant type taken at Arlington, Maryland, but have not as yet determined the inheritance of the character.

Since this was written STURTEVANT'S catalog of the North American Drosophilae has appeared, in which these flies are described (STURTEVANT 1921). STURTEVANT'S mutant is named *Drosophila repleta*. The mutant scarlet, previously reported and the "variable" of this paper belong to a new species called *Drosophila hydei*. The third form is probably a new species named by STURTEVANT, *D. mulleri*.

ORIGIN AND DESCRIPTION OF VARIABLE

Variable was first noted January 13, 1917, among the offspring from a cross of the mutant scarlet to wild stock in which the behavior of the mutant scarlet in back-crosses was to be tested (bottle 10, table III, Proceedings Indiana Academy 1917). In a total of about 100 flies, twenty were found with eyes that varied in size from mere specks (or in fact no eyes), to eyes of normal size and shape. Since both scarlet and wild-type eyes were similarly affected it is highly probable that these mutants do not represent the first mutation of the gene. It is impossible to state whether the change originated in the wild stock or in the mutated scarlet stock.

The ommatidia of the affected eyes are missing from the outer border of the eye, leaving a light brown band encircling the reduced eye. This holds for eyes down to about half size. Below this stage of reduction the eyes are often irregular, the light area is apparently carried toward the center where the few ommatidia protrude as an irregular mass from a somewhat depressed area. When reduced to a speck, this lies slightly posterior to the center of the normal eye. The small-eyed animals have an arrow-headed appearance which is in marked contrast to the wild flies with their large eyes and blunt heads.

Speck eyes were selected and inbred for a few generations as were the flies with eyes about half normal size, but both have given descendants with practically the same range of forms. The stock has apparently changed, however, in another particular, for a comparison of the descendants with the mutants that originally appeared will show that the eyes of each affected individual are more symmetrical in size and shape. The eyes of the first twenty mutants were very asymmetrical. The eye color of the mature wild-type *repleta* is very dark and will be referred to throughout the paper as mahogany.

EARLY HISTORY OF THE BEHAVIOR OF VARIABLE IN INHERITANCE

A small amount of data is given here to show the type of complicated results obtained before the key to the situation was found. The twenty flies discovered on January 13, 1917, were made up in three different cultures, and to my great surprise only normal-eyed flies appeared among the descendants until April 30 when a count yielded 2 males and 4 females with eyes about one-half normal size, 15 normal-eyed males, and 15 normal-eyed females. On May 3, there appeared 45 with normal eyes, and 15 flies with eyes one-half normal size. The small-eyed forms were selected and produced 466 normal flies and 32 with eyes that varied in size from speck to normal. The small-eyed flies were again selected, and in the summer of 1917 a count from this culture gave 147 females, and 142 males with eyes varying from one-half normal size to eyes entirely missing. Crosses were made by my students with this stock during the winter of 1917-1918. Ignorance of the fact that temperature was a determining factor in the realization of the character, led us at the time to ascribe our conflicting results to lack of purity of the stock.

Crosses between the wild stock and variable, made on March 2, 1918, gave the following results: The wild-stock ♂ mated to the scarlet variable ♀ gave more than 400 offspring with eyes like those of the wild type. These when inbred produced 1554 mahogany normal, 35 mahogany variable, 331 scarlet normal and 38 scarlet variable.

The reciprocal cross in which the scarlet variable ♂ was crossed to the wild-stock ♀ yielded about 400 sons and daughters with eyes characteristic of the wild type. These when inbred produced 390 mahogany normal, 8 mahogany variable, 81 scarlet normal and 14 scarlet variable.

These crosses give a grand total of 1944 mahogany to 464 scarlet, a ratio of 4.3 to 1. The ratio usually obtained on crossing scarlet to the wild type is 3 wild type to 1 scarlet. It seems likely that variable has in some way modified the ratio.

There are 2356 normal-eyed flies and 105 variables, a ratio of 22 to 1, a result we can understand in part when we know that the temperature can shift such a result within very wide limits.

BEHAVIOR OF VARIABLE UNDER A CONTROLLED TEMPERATURE

The study was carried forward from this point in light of the fact that eyeless flies were more likely to appear at high temperatures than at low temperatures. During the summer of 1918 the speck-eyed form had appeared in large numbers. On October 5, there appeared in a culture bottle that had been made up on May 14, 30 flies with eyes that varied from one-half the normal size to flies with mere specks for eyes. Several cultures were made from this bottle and placed in a rudely constructed warm chamber. The temperature fluctuated between 25° to 30° C. Speck-eyed flies emerged by the hundreds. In fact the cultures were pure for this character, while the flies that emerged from the control bottles, kept at a reduced room temperature during October and November, had eyes indistinguishable from the normal type. This showed that temperature was at least one deciding factor in the realization of the new character.

That moisture plays some part in the expression of the character would seem to be indicated by the fact that invariably an increasing number of flies with reduced eyes appear as the cultures become dry. Moreover the speck condition becomes intensified in dry cultures. It frequently happens that the first flies to emerge from pure variable cultures that have been kept at a relatively high temperature, have eyes larger than those that emerge later. In fact the first flies that emerge may be indistinguishable from normal while later only speck-eyed forms appear. This is shown in table 1. The obvious interpretation is that development has been accelerated by the temperature so that the plastic moment in the life of the fly is passed before the temperature has brought about any change in the moisture conditions of the culture. Many changes are taking place in a fermenting mass of banana exposed to a temperature of 25° to 30° C, so that one can not be sure from the evidence at hand that temperature exerts its influence through a change in moisture. We can only assert that the eyeless condition is realized under warm dry conditions.

LINKAGE OF VARIABLE AND SCARLET

With a stock that was pure for variable as shown by a number of tests and with the fact established that the character could be controlled by subjecting the cultures to the proper temperatures, the behavior of

scarlet and variable in the same cross was determined. Wild-stock virgin females, whose history was known, were mated to the double recessive scarlet speck-eyed males and kept at room temperature. All of the hybrids were like the wild type. These were back-crossed to the double-recessive parent stock. The cultures were kept in the warm chamber with the result shown in table 2. This table lists the results from three such tests. The data would seem to indicate that the full temperature effects were not realized, but it is evident that there is linkage between scarlet and variable. No mahogany variable flies result from this combination, which is explicable on the assumption that the characters do not cross over in the male.

TABLE 1

This table shows the history of two culture bottles of pure variable, that were well supplied with food and kept at a high temperature. Note that the first flies to hatch have larger eyes than those that hatch later

CULTURE NUMBER	DATE EXAMINED, NOVEMBER, 1918	NUMBER OF FLIES	CONDITION OF EYE
1	9	10	Eyes normal size
	14	9	Slight reduction
	16	22	One-half size
	20	150	One-half size to mere specks
	22	100	One-half size to minimal
2	9	14	Slight reduction
	14	19	Slight deduction
	16	15	Three-fourths size
	20	100	About one-half size
	22	35	One-half size—a few minimal
	28	30	One-half size to minimal

The relation between scarlet and variable was also studied in the reciprocal cross in which the hybrid ♀ was back-crossed to the double-recessive scarlet variable ♂, under the same conditions as in the above test. From this combination all four classes appear as in table 3. This table lists the results from three separate tests. These results are consistent in showing that variable and scarlet are linked and that there is crossing over in the female and none in the male. The crosses, however, do not yield the ratios which the formula demands. This is probably due to lack of environmental control, although genetic factors may be involved. Certain difficulties are met in breeding this fly which make some of the problems here encountered hard to bring under control.

Tables 2 and 3 are compiled from records which show the sex ratio in each of the different classes to be near equality. The detailed records also show that in so far as the distribution of the characters from the back-crosses are concerned it makes no difference whether the double recessive entered the hybrid from the male or female.

TABLE 2

Back-cross. Offspring from scarlet eyeless ♀ × hybrid ♂ (Wild × scarlet eyeless). Cultures kept in warm box. The tables list the totals from three separate tests. The sex ratio for each of the different classes was near equality.

CLASSES.....	NORMAL MAHOGANY	NORMAL SCARLET	MAHOGANY VARIABLE	SCARLET VARIABLE
Test 1.....	1023	443	0	412
Test 2.....	16	3	0	2
Test 3.....	35	11	0	11
Total.....	1074	457	0	425

TABLE 3

Back-cross. Reciprocal of 2. Offspring from hybrid ♀ × scarlet eyeless ♂ (wild × scarlet eyeless). Cultures were kept in warm chamber. The sex ratio for each of the different classes was near equality. The data are compiled from three separate tests.

CLASSES.....	NORMAL MAHOGANY	NORMAL SCARLET	MAHOGANY VARIABLE	SCARLET VARIABLE
Test 1.....	876	634	103	271
Test 2.....	113	60	11	24
Test 3.....	63	22	2	7
Total.....	1052	726	116	302

STAGE IN THE LIFE CYCLE OF THE FLY AT WHICH THE ENVIRONMENTAL INFLUENCE IS EXERTED

The following experiment demonstrates that the environmental influence which determines whether the eye of the homozygous variable shall be large or small is exerted after the egg is fertilized and deposited. On January 1, 1919, 100 speck-eyed flies were taken from a pure culture of variable scarlet from the warm box. The selected flies were kept warm and were permitted to lay their eggs on filter paper to which a small piece of banana had been added. Every twenty-four hours the banana with the

eggs was placed in a separate bottle with fresh food and returned to the warm box to develop. On the eighth day of the test the eggs were divided into two lots, one lot was returned to the warm box and one was placed on the window ledge where the temperature at this time of year was rather cool.

A comparison of the flies that emerged on the corresponding dates as given in tables 4 and 5 will show that those subjected to the warm temperature had defective eyes, while the eyes of those that developed at the reduced temperature were like the wild type. These results are not to be explained in terms of viability. It is clear that temperature is a determining factor in the realization of the speck-eyed form, and that the influence is exerted after the egg is fertilized and deposited.

Table 4 brings out the fact that moisture plays a part in the realization of the speck-eyed flies. The first flies to hatch from the warm cultures have eyes larger than those that hatch later, that is after the culture begins to dry. The warm box in which the eggs were kept isolated during the first five days in January dropped somewhat in temperature due to extremely cold weather. On January 6, 1919, the eggs were transferred to a newly constructed chamber that was kept more nearly uniform at 28° C. The effect is shown in table 4, cultures 12 to 15. It would seem that when development is accelerated as a result of exposure to high temperature, the flies are likely to have large eyes, as in these cultures they approximate the normal size. If delayed, as in culture 8, until a certain drying effect is produced, the first flies to appear are likely to be of minimal size.

CROSSES INVOLVING DIFFERENT COMBINATIONS OF SCARLET AND VARIABLE AND THEIR NORMAL ALLELOMORPHS UNDER DIFFERENT TEMPERATURE CONDITIONS

A number of crosses have been made in order to determine whether or not the ratios are modified as a result of keeping the parents at one temperature and subjecting their offspring to another. The result is not conclusive as bearing on this point as the ratios obtained approximated those we had obtained during warm summer temperatures. The data, however, are given as substantiation of the claim as to the behavior of the character in inheritance. In some of these combinations flies genotypically eyeless but phenotypically normal were used as indicated in the table headings.

TABLE 4

The type of eye that results on subjecting the fertilized eggs of scarlet variable to a warm temperature. Compare with table 5.

DATE OF ISOLATION OF EGGS JANUARY, 1919	BOTTLE NUMBER	NUMBER OF DAYS OF INCUBATION, NUMBER OF FLIES EMERGED AND CONDITION OF EYES											
		Days	Flies	Eyes	Days	Flies	Eyes	Days	Flies	Eyes	Days	Flies	Eyes
1	1	16	35	¾							38		
2	2	15	22	¾							37		
3	3	14	20	¾							36	10	m
4	4	13	10	¾	16	7	¾	23	4	m	35		
5	5				15	4	½	22	11	m	34	5	m
6	6				14	0		21	1	m	33	20	m
7	7				13	0		20	0		32	10	¾
	7a				13	0		20	1	m	32	20	m
8	8				16	0		19	0	m	31	10	m
9	9				15	0		18	12	m	30	3	m
10	10				14	56	½	17	28	½m	29	13	m
11	11				13	0		16	0		28	0	
12	12				12	9	½	15	15	½+	27	0	
13	13				11	0		14	25	½	26	25	m
14	14				10	1	½	13	20	¾	25		
15	15				8	0		11	15	N−	23		

N =normal
N− =slightly less than normal
¾ =¾ normal size
½ =½ normal size
½− =less than ½ normal size
m =minimal, i.-e., mere specks

TABLE 5

The type of eye that results on subjecting the fertilized eggs of scarlet variable to a reduced temperature. Eggs laid by the same parents as in table 4. These eggs give rise to flies with normal eyes while the eggs used in table 4 give rise to defective eyes.

	JANUARY, 1919									
Date of Isolation	8	9	10	11	12	13	14	15	16	17
Number of flies emerged	21	8	13	1	1	0	50	10	30	4
Condition of eyes.	Normal	Normal	Normal	Normal	Normal	Normal	Normal*	Normal	Normal	Normal

*A few flies with eyes slightly smaller than normal.

Wild-stock ♀ × scarlet variable ♂ with normal eye

This combination made November 13, 1918, and kept warm, gave more than 100 flies like that of the wild type. Virgin F_1 flies were mated in pairs and returned to the warm box with result given in table 6.

TABLE 6

F_2 from wild-stock ♀ × scarlet normal-eye ♂ from eyeless stock. The cultures were kept in the warm chamber.

CLASSES.....	MAHOGANY NORMAL		SCARLET NORMAL		MAHOGANY VARIABLE		SCARLET VARIABLE	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
Total.....	281	301	53	84	8	4	8	7

Wild-stock ♀ × variable scarlet ♂ with very small eyes

The wild-stock ♀ crossed to the variable scarlet ♂ with very small eyes, made at the same time as the cross in the previous experiment, gave in F_1 over 100 flies all of the wild type. These inbred produced in the F_2, classes given in table 7.

Normal-eyed ♀ from variable scarlet stock × wild-stock ♂

Normal-eyed ♀ from variable scarlet stock × wild-stock ♂ mated November 12, 1918, gave 75 wild-type flies. These inbred and kept warm

gave in F_2 the different classes as shown in table 8. The F_1 was mated December 19, 1918.

TABLE 7

F_2 from wild-stock ♀ × variable scarlet ♂ with minimal eyes. Cultures kept in warm chamber.

CLASSES.....	MAHOGANY NORMAL		SCARLET NORMAL		MAHOGANY VARIABLE		SCARLET VARIABLE		REMARKS
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	
	9	28	6	3	0	0	0	0	
	40	34	6	11	1	0	3	1	
	70	67	8	8	0	0	1	0	
									147 normal 14 speck
Total.....	119	129	20	22	1	0	4	1	

TABLE 8

F_2 from normal-eyed variable scarlet ♀ × wild-stock ♂.

CLASSES.....	MAHOGANY NORMAL		SCARLET NORMAL		MAHOGANY VARIABLE		SCARLET VARIABLE	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
Total.....	44	41	9	10	1	1	0	1

Wild-stock ♀ × minimal-eyed scarlet ♂

The wild-stock ♀ was crossed to 6 minimal-eyed scarlet males November 12, 1918, and kept at cool room temperature. On December 31, 1918, over 100 wild-type flies appeared. These were mated in pairs and one set kept in the warm box and one in the cold.

The F_1 was mated December 31 and produced the classes shown in table 9.

TABLE 9

F_2 from wild-stock ♀ × speck-eyed scarlet ♂.

TEMPERATURE CONDITIONS	CLASSES.....	MAHOGANY NORMAL	SCARLET NORMAL	MAHOGANY VARIABLE	SCARLET VARIABLE
Warm.....	Total	801	189	9	42
Cool.....	Total	170	40	3	7

Normal-eyed scarlet ♀ from pure minimal mass culture × wild-stock ♂

This cross kept at cool room temperature gave 85 all wild-type flies. A pair of the F_1 mated January 2, 1919, produced on January 30: normal mahogany 168; normal scarlet, 12♂♂, 13♀♀; variable mahogany, 3♂♂, 1♀♀; and variable scarlet, 6♂♂, 3♀♀.

Wild-stock ♀ × normal ♂ from minimal

A wild-stock female was crossed with a normal male from minimal November 13, 1918, and kept at cool room temperature. The F_1 gave 150 all wild type. Pairs were subjected to warm and cool conditions and yielded as shown in tables 10 and 11.

TABLE 10

F_2 from wild-stock ♀ × normal ♂ from variable stock kept in warm box.

CLASSES.....	MAHOGANY NORMAL	SCARLET NORMAL	MAHOGANY VARIABLE		SCARLET VARIABLE	
	Both sexes	Both sexes	♂♂	♀♀	♂♂	♀♀
Total.....	2110	456	7	7	18	26

TABLE 11

F_2 from wild-stock ♀ × normal ♂ from variable kept at cool room temperature.

CLASSES.....	MAHOGANY NORMAL	SCARLET NORMAL	MAHOGANY VARIABLE	SCARLET VARIABLE
Total.....	100	22	1	0

OBSERVATIONS ON THE STOCK CULTURES OF VARIABLE

Among the normal-eyed descendants of the original bottle from which the 20 variable flies appeared there was found on April 7, 1917, a scarlet-eyed female with eyes about one-half normal size. This culture was under observation for the inheritance of a suspected wing mutation. This fly was mated to a somatically normal-eyed scarlet male from the same culture. The F_1 generation gave: 35 normal-eyed ♂♂, 40 normal-eyed ♀♀, 9 variable ♂♂ and 15 variable ♀♀.

The variable stock was established by selecting variable eye from the flies just listed. In June they produced 147 females and 142 males pure for variable. The eyes ranged from one-half the normal size to eyes almost entirely missing.

TABLE 12

Summary of the combinations made and classes realized, arranged to show that scarlet and variable are linked and that the characters do not cross over in the male.

SET OF EXPERIMENTS	P_1 COMBINATION	F_1	F_2 CLASSES			
I and III			Mahogany normal	Scarlet normal	Mahogany variable	Scarlet variable
	Variable scarlet ♀ × normal mahogany ♂	All wild type	384	109	18	13
			761	161	15	22
			793	170	20	16
			85	19	2	1
			127	20	1	3
			168	25	4	9
Total.....			2318	504	60	64
	Normal mahogany ♀ × variable scarlet ♂	All wild type	164	25	2	5
			226	56	6	9
			582	137	12	15
			248	42	1	5
			801	189	9	42
			170	40	3	7
			2176	479	15	51
			100	22	1	0
Total.....			4567	990	49	134
II	P_1		F_1 CLASSES			
	Back-cross hybrid ♀ × variable scarlet ♂		876	634	103	271
			113	60	11	24
			63	22	2	7
Total.....			1052	716	116	302
	Back-cross variable scarlet ♀ × hybrid ♂		1023	443	0	412
			16	3	0	2
			35	11	0	11
Total			1074	457	0	425

The normal-eyed flies of April 30 were made up in mass culture and produced 466 normal-eyed flies and 32 with eyes varying from almost none to normal.

A female from the purified variable stock that had mere specks for eyes was mated to wild-stock males on May 28, 1917. This gave during June 21st to 24th, 27 sons and 22 daughters, all with wild-type eyes.

These inbred produced in the next generation: 384 normal mahogany, 109 normal scarlet, 18 variable mahogany, 13 variable scarlet.

During the fall and winter of 1917-1918 the inheritance of variable was studied in different combinations of back-crosses, but most disturbing ratios appeared. An examination of the stock bottles showed that they had apparently reverted to normal. I attributed this at the time to contamination and in the spring of 1918 commenced once again to purify the stock by selection. In this I was apparently successful, for in the summer only speck-eyed forms were to be found in the cultures.

October 5, 1918. From bottle made up May 14, 30 flies with eyes fairly uniform, about one-half the normal size. This bottle was kept warm and small amounts of banana added from time to time. October 14, all very small eyes. October 21, 8 very small eyes. October 28, many flies with eyes one-half size to minimal. October 30, many one-half size and larger. November 4, many one-half size and larger; 2 minimal.

The bottle was now placed near the window in the cold and on December 12 all the flies had eyes of the wild-type size. The bottle was placed again in the warm chamber and on January 9, 1919, all the flies had very small eyes. A bottle of variable produced on November 5, 1919, 2 normal, later 10 normal and 18 normal. It was then placed in the warm box and on December 7, 20 flies, with eyes one-half size and less, appeared, later 21 flies all with mere specks appeared. A bottle of minimal-eyed forms was made up October 8 and kept at cool room temperature. It produced: November 11, 36 normal. November 13, 12 normal. November 15, 27 normal. November 27, 27 normal and one slightly less than normal.

During the fall and winter of 1918-1919 experiments were carried out as recorded in tables 2 to 9. The key to the production of eyeless was evident. It could be made to appear in warm dry conditions.

A bottle made up from minimal-eyed forms on October 8, 1919, was kept at cool room temperature until November 4, 1919. It was then placed in the warm box. The larvae were good size when placed in the box. November 14, 5 normal. November 15, 10 normal. November 16, 16 normal. November 18, 13 less than normal. November 20, 4 one-half and less than

normal. November 22, 3 one-half and less than normal. November 26, 4 one-half and less than normal.

Two bottles kept at cool room temperature and dry (that is, no great excess of food was added). Made up October 19, 1919. Bottle 1 produced: November 27, 36 slightly less than normal. December 10, 25 slightly less than normal. Bottle 2 produced: November 27, 9 slightly less than normal. December 10, 40 slightly less than normal.

The stock at present is being subjected to a more exact environmental control.

RECORD OF TWO EYE MOSAICS

A mosaic male with the left eye scarlet and the right eye of wild-type color was found on March 12, 1919, from the descendants of a cross between the wild-stock male and normal scarlet variable female. The anomaly did not recur among the descendants and the inheritance was such as to show that the male was genotypically mahogany.

The second mosaic was a female that appeared October 19, 1920, with the lower third of the right eye covered with an irregular patch of scarlet; the remainder of the eye was mahogany, as was the left eye. This mosaic came from a mixed culture of mahogany and scarlet. No variables were present at this time. This fly was heterozygous; for, when mated to the scarlet male she gave 101 scarlet and 84 mahogany offspring.

DISCUSSION

A few cases in which an environmental stimulus caused marked phenotypic differences have been reported. In Primula BAUR (1911) showed that the plant transmitted the ability to produce red flowers at 15°C and white ones at 30°C. HOGE (1915) found that with a certain mutant of *Drosophila melanogaster*, reduplicated legs appeared in large numbers when subjected to a temperature of 9° to 10°C. ROBERTS (1918) reports that the length of the wings of vestigial is greatly modified by differences in temperature of 4° to 5°C. MORGAN (1915) has shown that the development of the abnormal abdomen in Drosophila is dependent upon moisture. KRAFKA (1920) in a very carefully planned series of experiments has recently shown that the number of facets that appear in the bar-eyed mutant of Drosophila is an expression of the temperature to which they are subjected at certain stages of development.

SUMMARY

A mutant, here called variable, of *Drosophila hydei* (Sturtevant) appeared in the same stock from which the mutant scarlet originated. These

are the only two mutants thus far reported in this species and they show linkage with no crossing over in the male.

The new character may appear in two different forms: (1) Full-eyed, indistinguishable from the normal eye, and (2) less than full-eyed, varying to mere specks.

The expression of the character, when homozygous, depends upon environmental conditions. Cultures of variable kept warm and dry produce speck-eyed forms in large numbers, while the cultures of variable kept cool and moist give rise to flies with normal-sized eyes. Temperature seems to exert its influence through its drying effect; for there is a marked tendency for dry cultures to produce speck-eyed forms. Newly made cultures of variable, subjected to a high temperature, in which the rate of development is greatly accelerated, are likely to produce eyes that approach the normal size.

There is an optimum temperature point beyond which development is so rapid that the eggs hatch while the culture is still wet even though at a high temperature. The flies that thus arise have eyes approaching normal size. On the other hand the temperature may be lowered and the culture become dry so that there is a tendency for a few small-eyed flies to appear even at a low temperature. A culture of variable kept moist and cold produces full-eyed forms, but when kept warm and dry, speck-eyed forms. It is clear that in so far as the expression of the character is concerned the influence is exerted after the egg is fertilized and deposited.

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LINKAGE RELATIONS OF THE SEX-LINKED CHARACTERS IN DROSOPHILA OBSCURA[1]

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INTRODUCTION

The cytological researches of METZ (1914, 1916) showed that there was considerable difference between the chromosome groups of various species

of Drosophila. It appeared that here was a good opportunity to test and extend the chromosome theory of heredity. The problem of working out the homologies of the chromosome groups in a genetic way to show the possible derivation of the different types of groupings is important. STURTEVANT (1921) has recently shown that there is near, if not complete, parallelism in the X chromosomes of *D. melanogaster* and a very similar species, *D. simulans*. By crossing mutants of *D. simulans* to mutants of similar appearance in *D. melanogaster* (*ampelophila*), STURTEVANT (1921) has been able to show conclusively that many of these are actually identical in that they act as allelomorphs and that their location in the chromosome agrees as to sequence and approximately as to "distance." These two species are very close to each other taxonomically and probably have the same chromosome complex.

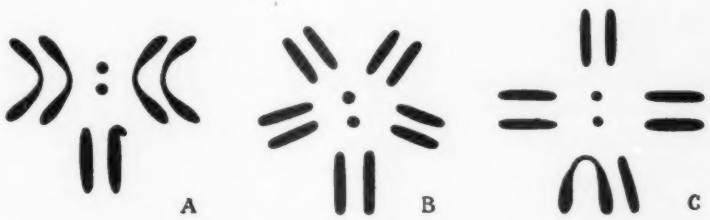

FIGURE 1.—Diagram of male chromosome groups of three species of Drosophila: A, *D. melanogaster;* B, *D. virilis;* C, *D. obscura* (after METZ).

Drosophila melanogaster, *virilis*, and *obscura* have somewhat similar chromosome groups which are shown for comparison in figure 1. These chromosome diagrams are based on the cytological study of METZ (1916) and show the male groups for the three species. The sex chromosomes are represented at the bottom of each figure. The study of *obscura* was undertaken with the hope that ultimately enough genetic evidence might be accumulated to show how the chromosome map in this species was related to that of the other two types on which extensive genetic data are available. The question of determining homology of chromosomes by comparison of similar mutants in species where allelomorphism cannot be determined by interspecific crosses, will be discussed in a later section. Our knowledge is still too limited to allow comparisons to be carried very far. However, the evidence presented in this paper justifies the belief that certain regions show parallelism and, furthermore, that a chromosome rearrangement has taken place.

An interesting feature about *D. obscura* is that the sex chromosomes are dimorphic, the X being quite long as compared with the Y. The Y is a rod similar in size to that in *D. melanogaster*, which differs in having a short hook. The long V-shaped chromosome, which is the X in *obscura*, contains about two-fifths of the chromatin of the haploid group at the metaphase stage. Whether the genetic phenomena would show any correspondence to this morphological length was an interesting question which it is now possible to answer. The present paper will be concerned largely with a description of sex-linked mutations and the working out of their linkage relations. This group shows such a large amount of crossing over, as to constitute a unique feature of the genetics of this species, and thus offers another confirmation of the chromosome theory of heredity.

STOCKS AND METHODS

Drosophila obscura agrees fairly closely in its general anatomical features with *D. melanogaster*. It is somewhat larger and is much darker, being grayish black rather than yellowish brown in color. The males have two sex combs on each front leg instead of only one, as in *melanogaster*, and in *obscura* the male abdomen is distinctly colored (red) by the testes. A description may be found in STURTEVANT'S (1921) monograph.

The work was started in the summer of 1919, using a wild-stock culture from the Pacific Coast which Dr. C. W. METZ had obtained several years earlier. This stock of *D. obscura* has now been kept going in the laboratory by means of mass culture transfers for upwards of five years, and during this time it has shown no noticeable decline in vigor or productivity. The species breeds more slowly than *D. melanogaster*, and takes fourteen to twenty days for a generation, depending on the temperature. The stock must have passed through at least eighty-five generations while under laboratory conditions. Later in the same summer (1919) another wild stock from Berkeley, California, was sent to Dr. METZ by Prof. E. B. BABCOCK, and this stock has also been used extensively.

The procedure adopted in breeding this species consists of making up matings, usually in pairs, and keeping the flies for two or three days in a vial before transferring to a half-pint bottle containing banana agar, seeded with yeast (BRIDGES 1920). In the vial, the females mature and are ready to lay their eggs as soon as they are transferred to the culture bottle. These bottles are kept until hatching in an incubator regulated to about 25°C. It is desirable for linkage experiments that all cultures be reared at nearly the same temperature, since the amount of crossing over perhaps varies with change of temperature, as PLOUGH (1917) has demon-

strated for *D. melanogaster;* however, the effect of change of temperature upon linkage has not yet been determined for this species. The count from a bottle may be continued with safety for twelve or fourteen days, but should be discontinued before the succeeding generation appears. Under ordinary conditions a pair may be expected to produce 150 to 250 offspring.

RESULTS

A summary of the results is given below and is followed by a brief statement and discussion of each point in the succeeding sections.

(1) *Drosophila obscura* mutates with sufficient frequency for genetic study.

(2) Non-disjunction of the sex chromosomes was not found.

(3) Crossing over between sex-linked characters is very frequent and genetically the X chromosome is over 170 units long.

(4) The number of linkage groups equals but does not exceed the haploid number of chromosomes.

(5) Some of the mutations seem to be identical with those in other species.

(6) The X chromosome of *obscura* appears to include a region not present in the X chromosome of *melanogaster*.

Mutability of Drosophila obscura

Drosophila obscura has produced over 40 mutants, a sufficient number to make it a promising species for genetic work. There have also appeared numerous variations which, although they doubtless have a genetic basis, yet are of little value in the study of heredity for one reason or another. Their most common fault is that the character may overlap the normal so frequently as to make an accurate classification impossible. Many mutants have a high degree of sterility, and still others only manifest themselves by a lethal effect, that is, individuals of a certain constitution do not survive. Mutation does not always produce extreme variations. The ones useful for genetic analysis are the ones sufficiently different to permit accurate classification.

Mutants have been found more frequently in certain species than in others. Within a given species mutations occur oftener in certain loci than in others. These same loci may mutate frequently in other species. However, loci which are very mutable in one species may not be so in another, and the second species may show mutable loci not known in the former. The number of mutants that are found depends largely on the number of individuals observed, and has no noticeable correlation to

season. The proportion of mutant genes which are dominant to their normal allelomorphs in *melanogaster* has been estimated as being about one in twelve, but it exceeds this in *obscura*, being about one in three, thus far.

Absence of non-disjunction

One of the proofs of the chromosome theory of heredity rests on the phenomenon of non-disjunction of the sex chromosomes of *D. melanogaster* (BRIDGES 1913, 1916). At the suggestion of Dr. C. W. METZ, I looked for non-disjunction in *D. obscura*. As many as 309 cultures that would have revealed non-disjunction if it had occurred were raised and inspected, but it was not found. If non-disjunction ever occurs in this species, it is probably less frequent than in *D. melanogaster*.

Frequent crossing over in the sex-linked group

The genetic length of the chromosome is obtained by adding together the percentages of crossing over between adjacent loci, and counting one percent as one unit of map distance. This gives a total of about 170 units for the X chromosome of *D. obscura*. Further data may very likely show that this value is only approximately correct. The amount of crossing over that occurs in the X chromosome of *D. obscura* far exceeds that in any other known chromosome. The question arises as to what is meant by a linkage group when such a high percentage of crossing over may be obtained. According to the chromosome theory of heredity, a linkage "group" consists of all the genes in one chromosome. Two genes, far distant from each other in the chromosome, might show free separation, but would still show very definite linkage to intermediate genes. Two genes which are far enough apart to have a map distance of 100 or more units will not be separated in every gamete but will only show a separation half the time, or even slightly less frequently. This may seem at first a paradox. The apparent contradiction is at once eliminated when one realizes that two breaks, or crossovers, may occur between two loci and, according to the method used, will be counted as two crossovers although no separation of the loci is produced. The frequency of separation does not go above 50 percent, even where the total amount of crossing over may mount up to 170 percent. The reason for this is that a single crossover between two genes will separate them, while a double crossover will result in an exchange of an intermediate group of genes that will leave the two in question still together. That the amount of separation between any two genes does not exceed 50 percent (even where the amount of crossing over

based on summation is calculated as far greater than this) is due to the fact that the relative frequency of double crossing over increases as the distance becomes longer. In a chromosome as "long" as the one worked with here, double crossing over may become as frequent as single crossing over, so that it cannot be determined from inspection of the crossover classes, which are single and which double. In some of the early three-point crosses, there was so little difference in size between the double, the two single, and the non-crossover classes that the sequence of the loci could not be determined. Discovery and use of other mutants which were intermediate in position helped to settle the difficulty. A four-point cross was made in which it was possible to determine the sequence of the four loci. This order has been repeatedly confirmed by many crosses made to determine the position of new genes in the group. A detailed discussion of the way in which the positions of the various sex-linked genes were determined in working out the X-chromosome map will be found in a later section. The amount of crossing over between the various loci, when summated, totals, as stated, 170 percent, which exceeds the genetic length of any other chromosome so far studied, by at least 50 units.

Correspondence in number of chromosomes and linkage groups

If the chromosomes are the bearers of the hereditary factors, and if a group of linked genes is composed of the genes borne by one chromosome, then the number of linkage groups may equal but cannot exceed the haploid number of chromosomes. The mutants so far found in *D. obscura* fall into five groups, and there are five pairs of chromosomes.

Identical mutations

The present paper contains descriptions of 28 sex-linked characters which are due to mutant genes in 23 different loci. At least 6 of these show striking resemblances to sex-linked mutants in other species, and in the case of three the evidence is strong that they are the same. Even though only a small proportion of the mutants obtained seems to be identical, nevertheless, the results so far obtained tend to support the view that the identification of chromosome groups in different species may be possible through further work.

It was realized that the matter of determining homology by relying on similar mutants in different species to mark corresponding chromosome regions was a difficult one, and capable of leading to mistaken ideas, because quite differently located genes may, by mutation, produce indistinguishable somatic conditions; and similar character-effects may be

produced by genes located in different chromosomes. For example, mutation of certain genes in the sex chromosome produces changes in either the eye color or wing shape very similar to the effects of mutations of certain genes in the autosomes. However, when two or more linked genes are found which stand in the same relative positions to each other in the two species and also produce the same somatic effects, the argument for regarding the genes as identical ones becomes very much stronger.

Chromosome rearrangement

The importance of the sequence of the genes may be shown by the following situation. Three closely linked genes in *D. obscura*, apparently identical with three in *melanogaster*, have been found on critical examination to have a different sequence in the two species. In *melanogaster* the order is yellow, white, notch; in *obscura* the order is yellow, notch, white. Had the sequence been the same in both cases, it would have seemed almost certain that the three characters were the same in each species. Then one must postulate some sort of a rearrangement, such as breaking off and re-attachment of a piece of chromosome. But it may seem more probable that one of the three is not the same character as the one it resembles in the other species. A more detailed consideration of this problem will be taken up later, after the various mutants have been described and their respective loci determined.

There are reasons for thinking that at least notch, white and eosin represent identical mutants, but there is a striking difference in their relative position in the two species in the chromosome map. In *melanogaster*, notch and white are closely linked and are both less than four units from the end of the chromosome map; in *obscura* they show a similar linkage value, but are at least 60 units from either end. These facts suggest that the size differences of the X chromosomes of the two species are due to the presence of a large piece of chromosome, attached at what corresponds to the left end of the *melanogaster* X.

DESCRIPTION AND LOCATION OF SEX-LINKED CHARACTERS

A multiple-allelomorphic locus and a modifying factor

Short (*s*)[2]

At the beginning of my work on *D. obscura*, Dr. C. W. METZ gave me a stock of a sex-linked recessive mutant named short. This mutant has

[2] In this section describing the mutants of the sex-linked group, the name of the character is followed by the symbol in parenthesis.

been described by him (METZ 1916). It is characterized by a shortening of the fourth and fifth veins, which fail to reach the wing margin. The effect on the fourth vein is variable, however, and this vein is sometimes normal. In flies with wings that are not damaged, the character can be classified with precision by means of the condition of the fifth vein, and it has been much used in the various crosses. Its value is increased by the fact that the mutant flies are vigorous and of good viability, and that the nature of the somatic effect does not interfere with classifying any other sex-linked character that is used in the same experiment. When other sex-linked mutants were found which made it possible to construct a chromosome map, it turned out that short occupied a locus at the extreme right end of the group, and that about 170 percent of crossing over occurred between it and the other end.

Shorter (s^r)

Shorter is a new mutant, allelomorphic to short, which appeared in culture 206, October 19, 1919. The mother was known to carry the gene for short in one chromosome (derived from her mother) and she should have received a normal allelomorph in the other chromosome from her father which was normal. However, she produced 40 sons which were short and 50 of more extreme type which were called "shorter." Later crosses of shorter males to short females produced daughters with the venation of the wings always affected. This showed that the two genes were allelomorphic. The gene for shorter occurred as a mutation from the normal gene. This mutant can be distinguished from short in that shorter affects all the longitudinal veins, second to fifth, and the gap by which they fail to reach the margin is greater than in short. The viability of shorter is good, and the character is infallible in its appearance and quite easy to classify.

Shortest (s^s)

The third mutant allelomorph in the short locus appeared in culture 329 as a single male, November 21, 1919. This was in the second count of the bottle, and no more "shortest" were obtained in the three subsequent counts. A total of 81 males were produced by the mother of this culture, which was homozygous for short. From this it is evident that shortest occurred as a mutation in the short gene in a maternal X chromosome either during meiosis or shortly before. In appearance, the shortest male is similar to the shorter male but more extreme. The longitudinal veins

(second to fifth) of the wings are more shortened, and in some cases do not reach more than half way to the distal margin. The range of variation of shorter and shortest overlaps, so that they could not be separated from each other in all cases if used in the same experiment. In general, shortest males are distinctly more extreme than shorter males; so that in two homozygous populations the difference is easily discernible. This difference in range between the males of the two types does not hold for the females, which are quite similar in appearance. Their ranges of variation seem to coincide, although it is probable that biometrical methods would show the two curves to be not identical for the two types of females. The mutant shortest shows sexual dimorphism in that the males are more extreme than the females.

Dominance relations of the short allelomorphs

The normal gene is completely dominant to each of the three mutant allelomorphs in the short locus. By crossing short to shorter or to shortest, females were obtained which were the short-shorter or the short-shortest compounds.[2] In both cases the compound females looked like homozygous short females, and were not intermediate in condition. Short behaved as a dominant to both of its more extreme mutant allelomorphs. The shorter-shortest compound looked like homozygous shorter or shortest females, which have been already described as indistinguishable in appearance. Consequently, there is no question of dominance involved.

Gap (*g*), a disproportionate modifier of short

In the summer of 1919, Dr. METZ informed me that he had observed occasional flies in his cultures which had a gap in the fifth longitudinal vein, that is, the vein did not reach entirely to the margin of the wing. Such flies looked like extreme variants of short toward normal. Breeding tests showed that gap is inherited as an autosomal recessive belonging to the third linkage group, and in a homozygous condition acts as a modifier of short. Gap flies always have the fifth vein slightly shortened, and may or may not have a slight gap in the second vein.

Gap acts as a disproportionate modifier of short. BRIDGES (1919) has used this term to designate cases where the character shown by the double mutant type is different from what would be expected from the combined characters of the single mutant types. Flies which are homozygous for both gap and short have the second to fifth longitudinal veins of the wings shortened so that they do not extend to the distal margin. Such individuals are similar in appearance to shorter. Since the third vein is not affected

by either gap or short alone, it was not anticipated that this vein would be distinctly shortened in the double recessive, and yet such is the case. For this reason, gap is considered a disproportionate modifier. It is a recessive modifier, since it acts only in the homozygous condition.

Gap and heterozygous short

Females which are heterozygous for short and homozygous for gap appear like ordinary homozygous short females, and were thought to be such when first observed. These short females were believed to be exceptions to the ordinary rule of sex-linked inheritance and to have occurred by non-disjunction of the X chromosomes of the mother. Breeding tests showed such females were only heterozygous for short, and that their appearance was due to the presence of gap in a homozygous state. This means that short, which is ordinarily recessive, does produce a distinct effect when heterozygous in the presence of homozygous gap. The latter condition makes short semi-dominant. A somewhat similar case in this respect was described by the author (LANCEFIELD 1918) for *D. melanogaster*, where a recessive mutation was semi-dominant in individuals homozygous for a specific modifier.

Gap and short-shorter or short-shortest compounds[3]

Gap does not make either shorter or shortest individuals more extreme, but it acts on the compounds of short with shorter or shortest just as it does on homozygous short. Owing to the dominance of short, these two compounds resemble short females, and are similar in appearance to each other. In other words, gap affects the compounds of short in the same way as it does short itself.

Beaded (*b*)

Beaded was found in culture 99, August 27, 1919. The culture produced six males which looked as though the margin of the wings had been regularly nicked. At intervals, the normal wing margin was left intact, and the dark bristles present in such areas formed isolated patches. The name "beaded" was applied to the mutant, but it is not the same as the autosomal dominant mutant of the same name in *D. melanogaster*. In culture 99, there were 72 females and 37 males produced, of which 6 males were beaded. This 2:1 ratio of females to males and the deficiency of beaded males can only mean that there was a sex-linked lethal in the same chromo-

[3] An individual which is heterozygous for two mutant allelomorphs in the same locus is called a compound.

some as beaded. The 6 males which appeared were crossovers between the lethal and the beaded genes. Three of these 6 were also crossovers with short, and were therefore double crossovers. The fact that 3 of the 6 beaded males carried a chromosome that had arisen by double crossing over indicated that beaded and short were far apart. It was impossible to say in which X of the mother the mutation occurred. From the ratio of sexes obtained and from the fact that the beaded males appeared in the second count and continued to appear to the end, it is evident that the mother of culture 99 was heterozygous for beaded and lethal. Her mother had produced a normal sex ratio. One other female from culture 13, out of 5 tested, had the same constitution as the one in culture 99, and this fact proves that the mutant genes beaded and lethal had been present in the parents. The data do not determine the stage at which the mutations occurred.

Locus and valuation of beaded

Since beaded was the second mutant locus obtained in the X chromosome, it has been extensively used in later experiments. Its viability is nearly as good as normal, and both sexes are vigorous and breed readily. The character does not overlap normal and can always be classified unless the wings are badly damaged. The beaded effect is more pronounced in the males than in the females. Beaded does not interfere with the classification of any other sex-linked character, and only one other masks the beaded character.

From the first appearance of beaded, it was evident that its locus was far enough away from short to give frequent double crossing over. In 5 cultures, to test the linkage of short and beaded, 531 males were obtained, of which 249 or 46.9 percent were crossovers. However, since the region is long enough for double crossing over to occur freely, and since a double crossover could not be detected in a two-point cross, the region between must be much longer than 46 units. The determination of the locus of beaded in the chromosome had to wait until mutations were obtained involving intermediate loci. Suffice it to say here, that beaded and short were later found to be more than 140 units apart.

Fused (f_u)

Fused males were noticed in culture 565 on January 31, 1920, and, in all, 24 males of this mutant type and 3 normals were obtained. The mother was a female with a break in the fourth longitudinal vein of the wing. This condition was due to a dominant sex-linked gene which also acts as a

recessive lethal and so kills the males of that genetic constitution. This mutant will be described later in this paper under the name of "broken." After the first fused males were observed, the culture produced 61 females and 27 males, which is in the ratio expected if half the males fail to survive. The great excess of fused males indicated that the gene for fused was in the opposite chromosome to broken (which is the lethal), and that there was about 12 percent of crossing over between the two loci. Since the mother in culture 565 received the X chromosome carrying broken from her mother, it follows that the X chromosome carrying fused was in the paternal one. The mutation to fused probably occurred in the germ track of the father. The exact stage cannot be decided.

Vermilion (*v*)

A vermilion-eyed male was found in culture 559 on January 30, 1920, and another on the tenth of February. The flies in the culture were taken out on February 7, and the second vermilion male hatched between that date and February 10. There was an interval of at least 8 days between the hatching of the two males. A single female was used as the mother of the culture. The number of males obtained from the bottle was not recorded, although 6 observations were made on the offspring to see if any stubby-bristle males would appear. The mutation to vermilion must have occurred in the mother of culture 559 at a late oögonial stage, as only a few gametes received the vermilion gene.

The color of the eyes of vermilion flies is lighter in shade and brighter than the normal color. The eye color of vermilion in *obscura* is strikingly similar to the autosomal eye color, scarlet, in the same species and in *D. simulans* and to sex-linked vermilion and autosomal scarlet in *D. melanogaster*, as well as to sex-linked vermilion in *D. virilis*.

Vermilion is a very valuable mutant and has been much used in placing other mutants in the chromosome. Eye-color mutants, in general, have good viability and, where the new color differs markedly from normal, are easy and certain of identification. Vermilion can be used in experiments with any other sex-linked mutants so far obtained except white. Vermilion was found to occupy a central position in the chromosome. Mutation has been especially frequent in that region. Vermilion, with short, beaded, and fused, was used in the first four-point experiment carried out, where the relative positions of these four loci were first determined. It early became evident that a four-point cross was necessary to determine whether vermilion was between beaded and short or at one end. The situation may be understood by considering the result obtained by crossing ver-

milion to beaded and short and obtaining the crossovers in F_2. Not anticipating any difficulty in detecting the double-crossover class, only three cultures were raised, and these produced 170 males. Since three loci are involved, there are two single-crossover classes and one double-crossover class, besides the non-crossover class, which may occur. The order, as determined by later crosses, is beaded-vermilion-short. Using this information to classify the kinds of males obtained, the 170 males were divided as follows: 39 non-crossovers, 34 crossovers in the first region, 60 in the second region, and 37 double crossovers. The three points were so far separated as to be nearly independent of each other.

Localization of genes in the chromosome

The relative position and distances of several loci were first determined with reasonable certainty by means of a four-point cross involving beaded, fused and short against vermilion. Four cultures produced 341 males which furnished information on the linkage relations. Only the males could be used, since the heterozygous females were not back-crossed to quadruple-recessive males, as these were not available at the time. The results of the cross are shown below. Inspection will show that one type of crossover class (beaded-vermilion-fused and short) is markedly smaller than the others.

TABLE 1

Nature of the cross: $\frac{\textit{beaded fused short}}{\textit{vermilion}}$

REGION	0		1		2		3		1,2		1,3		2,3		1,2,3		TOTAL
Class	*v*	*b* *fu* *s*	*b* *v*	*fu* *s*	*b*	*v* *fu* *s*	*b* *fu*	*v* *s*	*bv* *fu* *s*	+	*b* *v* *s*	*fu*	*b* *s*	*v* *fu*	*b* *v* *fu*	*s*	
	40	33	22	20	29	9	33	40	8	15	24	26	17	15	1	9	
Totals	73		42		38		73		23		50		32		10		341

On the basis of these data, the relative order of genes would be beaded, vermilion, fused and short in the order named. The observed amount of crossing over in this experiment is 36.4 percent between beaded and vermilion; 30.2 percent between vermilion and fused; and 48.4 percent between fused and short. A chromosome map constructed on the basis of these data, in which the loci are spaced according to these crossover values, is shown in figure 2. The observed crossover values in the three regions

totals 115 percent of crossing over, which is more than had been obtained in any case so far recorded. It is evident to anyone familiar with the phenomena of crossing over in other species of Drosophila, that all three of the regions here involved are long enough to admit of considerable double crossing over; consequently, the distances indicated in figure 2 will be lengthened by the use of intermediate loci.

The order of genes adopted in figure 2 based on the 341 males recorded above has been completely verified by many later crosses made up in other ways and involving many additional genes.

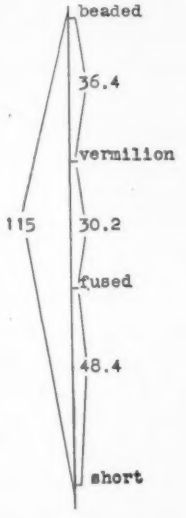

FIGURE 2.—Chromosome map based on cross involving beaded, vermilion, fused and short.

The frequency of triple crossing over obtained here is large. It is probably due to the length of the chromosome and the long regions involved.

Reduced (*r*)

Flies of both sexes which lacked the two anterior dorso-central bristles were first noticed in culture 465 on January 10, 1920. The name "reduced" was given to the mutant to distinguish it from an already existing mutant "minus" (not yet recorded in print) which looks like reduced, but is autosomal. Reduced behaves as a sex-linked recessive. Since reduced females, as well as reduced males, were obtained in culture 465, the father

must have been reduced and the mother heterozygous for the gene, and this is quite possible as they were brother and sister. They doubtless received the gene in the X chromosome from their mother, culture 339. However, since the occurrence of flies with missing bristles escaped observation in culture 339, no conclusion as to the precise origin of the mutation can be drawn.

Reduced flies differ from normal, typically, in the absence of the two anterior dorso-central bristles. Occasionally only one of these is gone, and more rarely a posterior one is absent. Since the stock has been homozygous, no fly with all four dorso-centrals present has been seen, although several hundred individuals have been examined. Reduced has been used in many crosses because its locus lies between vermilion and fused in a region where numerous mutant genes have been discovered. Its classification is accurate, though not easy, owing to the necessity of carefully examining the number of bristles. It can be used in crosses without interfering with the classification of any other sex-linked characters. Its viability is slightly below normal, and it hatches more slowly. The exact locus of reduced was determined in experiments involving rough. These crosses will be discussed after a description of the latter mutant.

Rough (r_o)

Rough was found as a single male in culture 605, January 19, 1920. The culture had been counted three times, and the rough-eyed male was observed in the third count. A total of 60 males was produced. These facts indicate that the mutation to rough occurred in the germ track of the mother of culture 605, and that only a few gametes (possibly only one) received the mutant gene. The roughened appearance of the eye seems to be due to the fact that the hairs among the ommatidia are longer and become more prominent than normal, so that the surface does not appear as even as in the normal fly. Rough has proved to be a dependable mutant to work with, and can be used with most other sex-linked mutants. Glazed and compressed interfere with the classification of rough, as they produce great changes in the texture of the eye. Rough is inherited as a sex-linked recessive. Its viability is good.

Loci of reduced and rough

Reduced and rough have been located in the linkage group by crosses involving vermilion and fused. A cross of reduced rough to vermilion was made, and five cultures produced 376 males which were used to determine the order and crossover values. Since it took a double crossover to

produce a vermilion reduced or a rough chromosome (the smallest classes), it is certain that reduced is between the other two in the chromosome. A further cross was necessary to determine whether reduced and rough were

TABLE 2

Nature of the cross: $\frac{vermilion}{reduced\ rough}$

REGION	0		1		2		1,2		TOTAL
Class	v	$r\ r_o$	$v\ r\ r_o$	+	$v\ r_o$	r	$v\ r$	r_o	
Total	315		23		30		8		376

on the beaded or the fused side of vermilion. Accordingly, a cross of reduced rough against vermilion fused was made. In 253 males obtained, 3 were crossovers between rough and fused, which indicates rather close linkage between these two loci. The number and nature of the crossovers obtained fitted best with a sequence of vermilion, reduced, rough, fused, and considerations from later crosses of different nature bear this out. Reduced and rough have crossed over 74 times in 742 individuals and thus show 10 percent of crossing over. By adding 1.2 percent (value for rough and fused) a total of less than 20 is obtained for the vermilion fused distance, which is too low as compared with the value previously obtained.

Notch (*N*)

A female appeared in culture 455, December 26, 1919, which was called "notch" because of its similarity in several features to the notch mutants of *D. melanogaster* and *D. funebris*. This female was mated to wild-type males. Only three offspring were obtained: a normal female, a notch female, and a normal male. However, this was sufficient to show that the notch gene behaved as a dominant, as it does in the two other species mentioned. Fortunately, the one notch daughter bred better than her mother, and, crossed to beaded short males in culture 602, produced 46 notch females, 48 normal females, and 40 males all normal. Later crosses involving notch females always produced similar results: presence of notch and not-notch females and only not-notch males, and in general, a ratio of females to males of 2:1. Notch is inherited as a sex-linked dominant in its somatic effect and also as a recessive lethal. The notch females are always heterozygous and the normal allelomorph allows them to survive. However, since males have only one X chromosome, all males receiving a sex-linked lethal die, since no normal allelomorph can be

present. This lethal action of notch operates to eliminate half the males and gives approximately a 2:1 sex ratio. The viability of notch females is poorer than that of normal ones, so that unless notch is run opposite to another mutant of poor viability, the notch females fall behind numerically, and there are not quite twice as many females as males. The notch mutant is named from one of several somatic peculiarities, namely, the posterior notching of the wings, particularly along the inside margin. A more reliable guide for identification consists in the thickening of the veins of the wings, especially noticeable at the juncture of the posterior cross vein and the longitudinal veins. The eight rows of microchaetae, or acrostichal hairs between the dorso-central bristles are disarranged and do not form definite rows as in the normal fly. The eyes may be somewhat roughened, though this can be made out in less than half the flies. The macrochaetae, in any part of the body, are frequently doubled, and on the scutellum one or more are often absent.

Other occurrences of notch

Notch$_2$ (N_2)

Notch mutations in *obscura* have occurred in three independent cases. The second occurrence of the notch mutation was discovered January 16, 1920, in culture 474, when two notch females were found. These two females, and also their progeny, showed the several somatic peculiarities of the original notch strain which was now designated as notch$_1$ to distinguish it from the second strain now established and known as notch$_2$. Notch$_2$ was undoubtedly a remutation of notch. The parents of culture 474 were a shorter female of shorter stock and a normal male of the Berkeley wild stock. The culture produced 105 daughters and 100 sons, which is sufficiently close to numerical equality of the sexes to show that the mother was not genetically notch. The notch females were found in culture 474 in the fourth or next to the last count of the bottle. They could not possibly have been due to contamination, as notch$_1$ was at this time being used for the first cross. The latter culture did not contain shorter, whereas both the notch$_2$ females from 474 were found to be heterozygous for it. If the notch mutation had occurred in a maternal X, the gene for shorter would be in the same chromosome as notch and if it had occurred in the paternal X, in the opposite chromosome. The progenies produced by these two females are recorded in table 3. It will be observed that in both cultures the number of shorter males slightly exceeds the number of normal males. One of these classes is a crossover class between notch

and shorter. It is known from later experiments, involving intermediate loci, that the total amount of crossing over (as obtained by summation between notch and shorter) amounts to at least 90 percent. However, even in such a long distance the relatively greater frequency of double crossing over reduces the observed percent of separation, in most instances, to below 50. It is probable then that the 72 normal males represent the crossover class. From this it follows that notch and shorter were probably in the opposite chromosomes, and since shorter came from the mother, then the mutation to notch occurred in the father, and at least two sperm carried the notch gene. Ordinarily, the notch gene is never transmitted by the sperm, because males which are genetically notch are not obtained.

TABLE 3

CULTURE	N_2 ♀	WILD-TYPE ♀	SHORTER ♂	WILD-TYPE ♂
604	73	99	51	46
603	36	52	29	26
Total.....	109	151	80	72

Only when the mutation itself takes place in the germ track of the male can the gene come from him. Notch$_2$ has been extensively tested genetically and behaves as a sex-linked dominant for its somatic effects and also as a recessive lethal.

Notch$_3$ (N_3)

Notch$_3$ is known only from a single female which was found in culture 1101, June 12, 1920. The female showed the several peculiarities already described for notch$_1$ including the effect on the wings, veins, and bristles. She died without progeny. The occurrence of notch in this culture could not be due to contamination, since the notch$_3$ female was yellow as well as notch, and half her brothers and sisters were expected to be yellow. On the other hand, there were no yellow notch flies which could have contaminated the culture, as the yellow notch combination had not yet been obtained from other sources.

Locus of notch

Only notch$_2$ has been accurately placed in the linkage group, as notch$_3$ did not breed and the notch$_1$ strain died out before the proper stock was available to use in a three-point cross with yellow and white. In the

crosses to yellow and white made individually, notch$_1$ showed close linkage to both. Notch$_2$ was crossed to reduced rough and the data derived from three cultures are summarized in table 4. Notch was shown to be to the left of both reduced and rough, since the rough males were clearly double crossovers. Since the amount of crossing over between notch and reduced is decidely more than that obtained between vermilion and reduced, the notch gene is assigned to a locus to the left of vermilion, or

TABLE 4

Nature of the cross: $\frac{notch}{reduced\ rough}$

Females	$r\ r_o$ ♂	+ ♂	r ♂	r_o ♂	Total ♂
248	71	29	11	2	113

between vermilion and beaded. This location of notch to the left of vermilion agrees with the results of later experiments. The locus of notch was more definitely determined by a cross to yellow vermilion. Yellow is a recessive mutation affecting the body color, and will be described in a

TABLE 5

Nature of the cross: $\frac{notch}{yellow\ vermilion}$

REGION.....	0	1	2	TOTAL
Class.....	$y\ v$ ♂	v ♂	y ♂	
Total.....	162	15	11	188

succeeding section of this paper. The summary of this cross appears in table 5. The absence of a double-crossover class leaves no doubt that the notch locus is situated between those for yellow and vermilion, and yellow is assigned to a locus to the left of notch, that is, between notch and beaded. As work progresses, it becomes evident that yellow and notch are not situated in close proximity to the left end of the chromosome map as are the yellow and notch of *melanogaster*. The more accurate placing of notch will be made after describing the yellow body color and the white and eosin eye-color mutants.

Deficiency nature of notch mutations

The nature of the change involved in the notch mutation in *Drosophila melanogaster* was studied by MOHR, who reported some interesting con-

clusions (MOHR 1919). It will suffice here to state that notch acts in some respects as a deficiency of an entire region and that the extent of the region affected varies in the different notch mutations of *melanogaster*. In the case of notch$_8$, the region extended as far as the white locus and acted as a deficiency for white. The phenomenon of deficiency was earlier discussed by BRIDGES (1917). In *D. obscura*, neither notch$_1$ nor notch$_2$ acts as a deficiency for white or its allelomorph, eosin. The color of the eyes of notch females which are heterozygous for white does not differ from the normal color. Notch$_2$ seemed to act as a deficiency for scutellar, a recessive mutation that removes some of the scutellar bristles. It was noticed that notch$_2$ females when heterozygous for scutellar had bristles missing on the scutellum more frequently than did notch females not carrying a gene for scutellar. However, notch$_2$ is not a deficiency for scutellar which is on the opposite side of yellow, and their combined behavior in removing scutellar bristles is probably better explained on the view that heterozygous scutellar strengthens the tendency, that notch mutations in *obscura* show, to remove some of the bristles.

Specific notch modifiers

In the case of *Drosophila melanogaster*, MORGAN (1919) has demonstrated the existence of genes which modify the character "notch." He found that these modifiers acted in different ways so that the notch condition was either increased or diminished according to the particular gene present. He was able to determine in which chromosomes the modifying genes were located.

In *D. obscura*, the condition of notch females in two instances has been strikingly changed due to the specific effect of modifying genes. Both would come in BRIDGES's (1919 a) category of specific modifiers, since the genes produce no visible effect except in notch individuals.

Achaete modifier

In May, 1920, it was observed that some of the notch females in two inbred sister cultures, 1021 and 1025, were almost denuded of bristles. The parents of these two cultures were notch$_1$ females from culture 958 mated to brothers, and neither of the notch mothers was observed to differ from the typical condition of notch. Had they been achaete, as were some of their daughters, the condition would almost certainly have been noticed. The achaete individuals were always notch, although 258 offspring were produced and the not-notch out-numbered the notch by 2 to 1. The

achaete notch$_1$ females were difficult to breed and the location of the modifying gene was not determined. The gene was shown to be recessive by out-crossing one achaete notch female. None of the notch daughters were achaete.

Split modifier of notch

A second notch modifier was observed in the case of notch$_2$, November 18, 1920, in culture 1481 when a notch$_2$ female appeared with the wings considerably shortened and split about half way in from the distal end. There is a separation along this split so that a crude Y shape is assumed by the wing, that is quite conspicuous. The only flies which are affected are the notch females. Split notch$_2$ females are markedly below the wild type in vigor and productivity, but one mating (1577) was successful in which such a female was out-crossed to a minute-bristle male. Minute is an autosomal dominant mutant. Some of the split notch offspring were also minute-bristle which showed that the modifier was a dominant. No fly showed the split-wing condition which was not also notch. Split must then be considered as a specific modifier. This was again demonstrated by using a minute-bristle son from this culture, which also carried the modifier, to cross to a notch female from a stock that was known to be without the modifying factor. The offspring from this mating (1602) were 49 notch females, 47 not-notch females, and 12 sons. Although the minute-bristle father used here had normal wings, he carried the gene for split, as 26 of his 49 notch daughters showed the split-wing condition. Although half of the 47 not-notch daughters and of the 12 sons carried the modifying factor, they did not manifest any indication of it. The behavior of minute and split in this culture showed that they were carried in the same pair of chromosomes, and split is, therefore, a member of the third group of characters. This conclusion is based on the observation that none of the 26 split notch females were minute-bristle, while all the 23 unmodified notch females were also minute.

Yellow (*y*)

Mutation to yellow has occurred twice; first on March 24, 1920, when a few yellow males were obtained in the slender-bristle stock. The body, bristles, and wings are yellow in color and differ very strikingly from their dark gray color in the wild form.

Yellow$_2$ does not differ in appearance from the first yellow mutant. It arose through independent mutation from normal. Two yellow males appeared in culture 1040 in the third count, May 30, 1920, and two more

in the fifth count, June 3. None appeared in two subsequent counts. Two recessive autosomal characters, scarlet and bithorax, were present in culture 1040. One yellow male was both scarlet and bithorax, while a second yellow male was bithorax. This is clear proof of the independent origin of yellow$_2$. Four yellow males were obtained in culture 1040 among 178 offspring, and these four came during the middle of the count. The mutation probably occurred in a maternal X chromosome not many divisions prior to maturation. Both yellows were inherited as sex-linked recessives, and by crossing were shown to be allelomorphs. The determination of the locus of yellow to the left of notch and vermilion has already been discussed. Yellow is a valuable mutant because of the ease and accuracy of its classification, its possible identity with similar mutants in other species, and because it can be used with any other sex-linked mutant. Its viability is fair. Yellow in *obscura* does not occupy the extreme end position in the chromosome map, but in order to make comparison easier, it is treated as the zero point, and map distance based on linkage is computed in both directions.

White (w_1)

The first mutation to white occurred in culture 641, when a white-eyed male was obtained February 13, 1920, in the second count of the bottle. Since no other white individuals were obtained in later counts, the mutation of a wild-type gene to white must have taken place in one of the X chromosomes of the mother at a late stage. The most conspicuous effect of the gene for white is the color of the compound eye and of the three ocelli. The white eyes have a grayish tinge, due probably to the hairs between the ommatidia, and are more similar in appearance to white flies in *D. melanogaster* that have an ebony body color than to the ordinary *melanogaster* white. The striking coloration of the abdomen in males of *D. obscura*, due to the orange color of the testes, is absent in white males. There is no such effect in the females, as the ovaries do not color the abdomen. White females have not produced offspring when mated to white or to any other males.

White$_2$ (w_2)

The second mutation to white took place in the reduced stock 592/2 where a few white males were found March 2, 1920. These white$_2$ males were similar in appearance to white$_1$, and the two were demonstrated to be allelomorphs when a white$_1$ male was mated to a female heterozygous

for white$_2$, and white daughters were obtained. The independent origin of white$_2$ was demonstrated by the fact that it arose in the reduced stock, and the white$_2$ males were also reduced. There was no white in the pedigree of the reduced stock, and had these white males arisen by a contamination, they would not have been reduced.

White is a valuable mutant because of its possible identity with white in *D. melanogaster* which was strengthened by the finding of eosin, an allelomorph to white. The fact that white showed very close linkage to both yellow and notch added interest. It will be recalled that white eye in *melanogaster*, found by MORGAN in 1910, was one of the first Drosophila mutants discovered. White in *D. obscura* has poor viability and hatches later than normal. The females are sterile. For genetic use, white is not as valuable as eosin, as the presence of eosin does not prevent the classification of vermilion; and further the eosin females are fertile. Since white shows only one percent of crossing over with notch, the two mutants are kept in the same stock by mating white males to notch females, heterozygous for white. Since the white females present do not produce offspring even if they do mate with the white males present, the stock is automatically balanced for notch and white. The stock is occasionally examined to make sure that wild-type males have not arisen by crossing over.

Eosin (w_e)

Males with eosin-colored eyes were obtained in culture 1310, August 12, 1920, and were called eosin on account of their similarity to eosin in *melanogaster*. The *obscura* eosin have a darker tinge to the eye, as is also true for white. The mother of culture 1310 was a notch$_1$ female and the gene for eosin must have been in the opposite chromosome from that carrying notch. Judging by the count obtained, 104 females, 9 eosin and 1 wild-type male, a sex-linked lethal other than notch was also present. In order to get a sex ratio of 10 females to 1 male, the other lethal should give about 20 percent of crossing over with notch, as only half the crossovers will be free from a lethal. The exact location of the lethal was not determined. As the notch$_1$ female was heterozygous for eosin opposite notch, it is evident that the mutation to eosin occurred in the paternal X chromosome.

Eosin is intermediate in shade between white and wild-type. It is a dull reddish orange color. The color of the eye differs in the two sexes, being markedly darker in the females than in the males. This sexual dimorphism agrees with that in *D. melanogaster*, and strengthens the prob-

ability of the two being identical mutants. The ocelli also have the eosin color. The male abdomen is not colored by the testes.

Eosin is also a valuable mutant because of its close linkage to yellow and notch. It has much better viability than white and is more generally useful. It behaves as a sex-linked recessive and its classification is easy and certain.

White and eosin, with their wild-type allelomorph, form a multiple-allelomorphic series. White and eosin were shown to be allelomorphs when the white-eosin compound was obtained. Such females are intermediate in eye color and are a shade lighter than eosin males, which are lighter than eosin females. A similar relation holds for white and eosin in *melanogaster*.

The location of yellow, notch, and white or eosin

Preliminary crosses involving two loci at the same time indicated that yellow, notch, and white-eosin were closely linked. This was of interest because the three similar mutants in *D. melanogaster* are also closely linked. However, the sequence, as determined by a three-point cross, proved to be different. The cross was made up using eosin against yellow notch$_2$. A summary of the results is given in table 6. Inspection of the

TABLE 6

Nature of the cross: $\frac{yellow\ notch_2}{eosin}$

	FEMALE							MALE		
Region.....		0		1		2		0	1	2
Class.....	Not classified	*y* N_2	*w*^{e}	*y w*^{e}	N_2	*y* N_2 *w*^{e}	+	*w*^{e}	*y w*^{e}	+
	321	201	167	7	11	1	4	274	12	5
Total.....	321	368		18		5		274	12	5

In this summary, the 321 females, not separately classified, occurred in cultures when a wild-type male was used. Only the males of such cultures could be used for crossover determination.

crossovers obtained shows the complete absence of representatives of a double-crossover class. The missing class would be yellow or notch eosin. The sequence is yellow notch eosin, which does not agree with the sequence in *melanogaster*, namely, yellow eosin notch. The result was a surprise, and while the above data seemed convincing enough, the cross was repeated in another way, using yellow eosin in one chromosome

against notch$_2$ in the other. The results obtained in the second cross confirmed those obtained in the first. Four cultures are summarized in table 7. Here the class yellow or notch eosin, which was missing in the pre-

TABLE 7

Nature of the cross: $\frac{\textit{yellow eosin}}{\textit{Notch}}$ ♀ × *yellow eosin* ♂.

	FEMALE						MALE			
Region.....	0		1		2		0	1	2	1,2
Class.....	*N*	*y w^e*	*y N*	*w^e*	*y*	*N w^e*	*y w^e*	*w^e*	*y*	+
	214	175	10	9	5	1	152	14	1	0
Total.....	389		19		6		152	14	1	0

vious cross, is now present, while the triple-mutant and the wild-type class are absent. Both crosses indicate the same sequence, namely, yellow notch$_2$ white (or eosin). A consideration of the difference in sequence as compared with that of the similarly named mutants in *D. melanogaster* will be found in a later section of this paper.

Having determined the sequence, the map distances may be calculated, using all the available data for these two regions, which is given in the regional summary. A yellow notch distance of 5 units is obtained, and a notch white (or eosin) distance of 1 unit. These three loci have their position in the linkage group to the left of vermilion. The yellow vermilion distance is about 14 units, with notch and white between. The sequence of the genes in the chromosome map is now beaded, yellow, notch$_2$, white (or eosin), vermilion, reduced, rough, fused, and short.

Scutellar (s_c)

The absence of some of the scutellar bristles on some males in slender-bristle stock was observed April 26, 1920. The character scutellar was somewhat variable as regards the absent bristles. Commonly, the two posterior bristles of the scutellum were missing, but occasionally they were present, while one or both of the anterior scutellar bristles were absent; and less frequently all four were gone. The post-vertical and ocellar bristles of the head, which are removed in the *melanogaster* scute, are not absent in this case. Judging from the observations made on the homozygous stock of scutellar, the character always shows itself. Scutellar is inherited as a sex-linked recessive.

Locus of scutellar

The gene for scutellar was located in the chromosome mainly by crosses involving yellow. The linkage between these two loci was very strong, as only four crossovers were obtained in a total of 1268 individuals. This gives 0.3 percent of crossing over. In order to determine whether scutellar is located to the right or left of yellow, a cross was made using yellow scutellar in one chromosome against eosin in the other. A summary of the result follows. Only the males were used for linkage. Evidently, scutellar eosin and yellow are single crossovers between scutellar and yellow. The

TABLE 8

Nature of the cross: $\frac{\textit{scutellar yellow}}{\textit{eosin}}$

Region	0		1		2	
Class	$s_e\ y$	w^e	$s_e\ w^e$	y	$+$	$s_e\ y\ w^e$
	166	141	2	0	12	3
Total	307		2		15	

two scutellar eosin flies constitute the critical crossovers. Both were tested by mating them individually to two virgin scutellar females to see whether they were genetically scutellar. In both cases, their scutellar constitution was demonstrated by the production of daughters which were uniformly scutellar. There is, thus, no doubt that the scutellar locus is to the left of yellow, that is, on the side away from notch.

Scutellar is a good workable mutant, but is not used very extensively, as its locus is so close to yellow which is more easily and accurately classified. Bristles may occasionally be missing from the scutellum in flies which are not genetically scutellar. Both sexes are vigorous and productive and the viability is good.

Pointed (*P*)

A female with long narrow wings, pointed distally, was observed in the third count of culture 482, January 10, 1920. This was the only individual of the kind obtained in the culture which produced 152 flies and was counted five times. In places where the wing is reduced, the heavy margin is absent as though it had been trimmed away. The typical condition of pointed-winged flies is very similar to the picture of beaded in ***melanogaster***, which is published in "The physical basis of heredity" by MORGAN

(1919) opposite page 258, although there is probably no genetic identity between the two. Since only one pointed fly was found in 152 offspring (although pointed is inherited as a dominant and would show itself in individuals which received the gene), it is reasonable to conclude that the mutation occurred at a very late stage, either during or shortly before maturation. There is no way of determining in which parent the mutation occurred.

Pointed is a dominant which is unfailing in its appearance, and for that reason useful. Its locus was found to be to the left of beaded, at the end of the chromosome map, to which it added about 20 units of length. Its classification is rapid and accurate. One thing mars its usefulness, namely, the fact that the pointed condition makes it impossible to classify for beaded, which is so located that it is frequently desirable to use them together. Since the reduction of the wings affects the venation, the wings are easily crumpled. It is difficult to classify for short or fused in pointed flies. Both sexes have excellent viability and good productivity.

Locus of pointed

Pointed was assigned an approximate locus in the chromosome map to the left of beaded, or on the side away from yellow. A critical cross of pointed to beaded vermilion was made after pointed had been crossed to various sex-linked mutants and had shown apparently free crossing over to all. Pointed and beaded were not crossed until as a last resort for the reason that the presence of the pointed condition made it impossible to determine whether an individual was also beaded or not. However, the linkage of the two mutants could be estimated in the cross of pointed against beaded by doubling the normal class, since, as stated, its reciprocal crossover class, pointed beaded, could not be distinguished from pointed. As the viability of the normal individuals is higher than that of the beaded, the result obtained by doubling the normals will be somewhat too high.

The males in three cultures of the cross of pointed against beaded vermilion were classified as in table 9. Of the two classes, wild-type and vermilion, which contain neither pointed nor beaded, one is a single- and the other a double-crossover class. The discrepancy in size of the two immediately suggests that the wild-type is the single-crossover class. Now, the reciprocal class of normal, or the pointed beaded vermilion flies, were counted among the pointed vermilion, and in the same way the pointed beaded were not separated from the pointed. It is clear that the pointed vermilion class outnumbers its reciprocal class more than does the pointed. If this is because the pointed beaded vermilion exceeded the pointed

beaded in numbers, it would confirm the view that the double-crossover class is represented by vermilion. By doubling the crossovers enumerated in the normal and vermilion classes, the percent of pointed-beaded crossing over obtained was 27.4.

TABLE 9

Nature of the cross: $\frac{pointed}{beaded\ vermilion}$

Class.....	*P* (*P b*)	*b v*	*P v* (*P b v*)	*b*	+	*v*	TOTAL
Total.....	52	40	52	32	21	7	204

Glazed (g_z)

The first count of culture 1163, July 2, 1920, showed the presence of two males with a new eye condition. The surface of the eyes was smoothed over, and had lost the slightly spiny condition of the normal eye. From the fact that only two males appeared and both in the first count, the origin of glazed may be traced to a mutation previous to maturation in one of the egg strings of the mother of culture 1163. Since one glazed male was vermilion and the other was not, there is no way to determine whether the mutation occurred in the maternal or paternal X chromosome.

Glazed is inherited as a sex-linked recessive. The viability of this mutant is a trifle below normal. The homozygous females have never produced any offspring even when used in mass cultures. The stock is kept going by mating glazed males to heterozygous females. Since the locus of glazed is close to that of pointed, it is fortunate that glazed can be used in crosses with either pointed or beaded without difficulty of classification. Glazed can also be used with any of the various eye colors. Aside from the sterility of the females, glazed has some distinct advantages over pointed, as it can be more generally used.

Locus of glazed

One of the original glazed males was yellow vermilion short, as well as glazed, and this quadruple recessive male was crossed to a wild-type female of Berkeley stock. From the results obtained from five cultures in this four-point cross, the locus of glazed seemed to be to the left of yellow. The other original glazed male was also yellow and short. It was mated to a fused female and three cultures were counted for linkage relations. The results here indicated the same location as had the preceding cross. It was evident that glazed was in the pointed beaded region.

Cross of glazed by beaded

A cross of glazed by beaded vermilion showed that the location of glazed, based on the two crosses already mentioned, was correct. This latter cross made it possible to determine the locus of glazed to the left of beaded, that is, on the same side as pointed. A glance at the summary of the two cultures will show that while 21 crossovers were obtained between beaded and vermilion, and 14 between glazed and beaded, there were no simultaneous crossovers in both regions. The sequence is glazed beaded vermilion. In crosses involving glazed and beaded 39 crossovers have occurred among 242 individuals, which gives a percentage of crossing over equal to 16.1.

TABLE 10

Nature of the cross: $\frac{glazed}{beaded\ vermilion}$

Region.....	0		1		2	
Class.....	*b v*	g_s	g_s *b v*	+	g_s *v*	*b*
	25	18	9	5	11	10
Total.....	43		14		21	

Cross of glazed to pointed

The previous crosses in which glazed has been used would indicate that its locus is not far from that of pointed, and a cross was made to get data on this point. Among 176 individuals, there were 7 crossovers, which gives 4 percent for a crossover value. Since the glazed beaded value plus the glazed pointed value together equal 20 (which is less than the estimated pointed beaded distance) glazed is tentatively assigned to a position between pointed and beaded. This is now in process of being tested by a cross involving three loci, simultaneously.

Broken (B_r)

During the third count on culture 418, two females were discovered December 18, 1919, which had a region of the fourth longitudinal veins missing, or much weakened. Later it was observed that the eyes of broken flies may be somewhat rough. The character is inherited as a sex-linked dominant in the females, but no broken male survives. Cultures raised from broken females show the same lethal ratio as has been described for

notch. The gene for broken may be assumed to act as a dominant for its somatic effect and also as a sex-linked recessive lethal. The origin of the mutant gene took place at a late stage in the germ track of one parent, whether of the father or the mother is uncertain.

Broken is not a valuable mutant, as the females do not always show their genetic constitution and were difficult to breed. The stock can be kept going only by using broken females.

Locus of broken

A cross of broken to vermilion rough showed that the locus of broken was intermediate between those two (see table 11). If broken is between vermilion and rough, it would take a double crossover to produce a wild-type male and none were obtained. Broken has not been used in a three-

TABLE 11

Nature of the cross: $\frac{broken}{vermilion\ rough}$

Class.....	$v\ r_o$	v	r_o	+
Total.....	77	7	5	0

point cross with reduced, and consequently, the relative position of these two genes is uncertain, although they are undoubtedly very close together. Based on the comparative linkage to vermilion, reduced is closer to vermilion than is broken. Between vermilion and broken, 55 crossovers occurred among 508 individuals, or 10.8 percent of crossing over took place. For vermilion reduced, a crossover value of 8.4 percent is obtained, based on 668 individuals. On these data, which are not conclusive, broken is assigned to a locus between reduced and rough.

Slender (s_l)

The character known as slender was discovered in culture 651, February 11, 1920. The mother used in the culture was evidently heterozygous for slender, as 17 of the sons were slender and 17 were not. Slender is inherited as a sex-linked recessive. The mutant was named from the change produced in the bristles, which are longer and smaller and more cylindrical than normal. They do not taper as the normal ones do.

Slender has not been used very much because it is located close to short which is somewhat easier to classify. Slender can always be distinguished from normal, and can be used with nearly all the other sex-linked mutants.

However, its classification is not rapid, as a careful examination is necessary in each case. The viability is good and both sexes are productive.

Location of slender

Slender was early crossed to short and found to give about 8 percent of crossing over with it. It was not determined which of the two occupied the end locus until crosses were made involving ascute and also compressed. These experiments showed that the sequence is compressed, ascute, slender, short. Ascute and short had been found to give about 22 percent of crossing over. This is approximately equal to the sum of the values obtained for ascute slender plus slender short. A four-point cross gave data confirming the location of slender between ascute and short.

Compressed (c_o)

In the first count, March 15, 1920, culture 803 produced four males of a mutant type which was called compressed. Such males were obtained throughout the time the culture was under observation and, in all, 36 compressed to 54 normal males were produced. The mother of culture 803 was heterozygous for beaded and short (in opposite chromosomes) as well as for the new mutant gene, compressed, but the compressed crossed over so freely with both that it could not be determined whether compressed was in the same chromosome with beaded or with short.

The mutant was named "compressed" because one of its most noticeable effects is a decided antero-posterior shortening of the thorax, so that the anterior dorso-central bristles, which are sometimes missing in this mutant, are back very close to the posterior ones. There frequently occurs a mid-dorsal depression on the thorax which is marked anteriorly. The small bristles, or acrostichal hairs, on the thorax are disarranged and do not run in regular rows as in wild-type flies. The reduction in size of the eyes is variable. Only a few ommatidia may remain, or all may be gone. Compressed individuals vary exceedingly in the difference in the condition of the two eyes. Cases are common where the eye on one side is very small and on the other side is nearly wild-type in size.

The poor viability of compressed causes it to run considerably behind wild-type in numbers, and, consequently, detracts from its genetic usefulness. It is located close to fused, which is a better mutant for most purposes. Compressed has some interest as being another case where a gene produces a number of diverse effects. It is inherited as a recessive.

Locus of compressed

The results obtained in culture 803, where compressed first appeared, made it evident that the gene for compressed was not near either to beaded or to short. Later crosses show close linkage with fused, as only 5 percent of crossing over was observed among 703 individuals. It has not been determined on which side of fused compressed is located.

Ascute (a_s)

A male was obtained in culture 1302, August 11, 1920, which resembled the autosomal mutant ascute of *D. melanogaster*, and an undescribed autosomal mutant of *obscura* known as grooveless. The new mutant was named ascute but as it turned out to be sex-linked, it probably is not identical with the autosomal scute of *melanogaster*. My records do not show how many ascute males were produced by culture 1302, as they were at first suspected of being a mutant already known, "grooveless."

In normal flies, there is a distinct groove separating the thorax and the scutellum on the dorsal side. This groove is absent in ascute. Ascute flies frequently show what appears to be an excrescence of brownish substance on each side of the body at the juncture of the thorax and the scutellum.

Ascute is a recessive. Its gene lies about 22 units from short, between fused and short. It is important in that it fills in the long gap to the left of short. The character can be identified in all individuals of that constitution. It can be used with any other sex-linked mutant without either being masked by the presence of the other. The viability of ascute is not good, as it usually runs distinctly below equality with the wild-type. The individuals are lacking in vigor and are short-lived.

Location of ascute

The first cross using ascute also involved pointed and yellow, and did not yield a very definite answer as to the locus of ascute except that it was a long way from either pointed or yellow. In this cross, 245 males were observed and the double-crossover class of 51 exceeded one of the single-crossover classes by 4. A cross using ascute short in one chromosome, against compressed in the other, gave a more definite result. Assuming that the 41 class is the double-crossover one, the sequence is compressed ascute short, and the ascute short distance is about 22 units. The crossovers between compressed and ascute amount to 44 percent of the total. A large amount of double crossing over doubtless occurs in such a

long region, but cannot be determined with accuracy until other mutants with intermediate loci are available to work with. In *D. melanogaster* a direct crossover value of 44 percent would indicate a map distance of approximately 60 units. However, it is not yet ascertained whether the observed separation frequency for long distances has the same relation to map distance in *D. obscura* as in *D. melanogaster*. Consequently, it is not entirely legitimate to correct for double crossing over in *obscura* with estimations based on the other species.

TABLE 12

Nature of the cross: $\frac{\textit{ascute short}}{\textit{compressed}}$

Region.....	0		1		2		1,2		
Class.....	c_0	a_s s	c_0 a_s s	+	c_0 s	a_s	c_0 a_s	s	
	95	91	50	98	26	27	13	28	
Total.....	186		148		53		41		428

Miniature (m_n)

A single miniature-winged male appeared in culture 1253, July 27, 1920. The mutation took place in the mother probably at a late stage in the formation of her gametes. The wings are normal in shape but are reduced to about two-thirds of the normal size and slightly darkened. The entire individual is smaller but not to the same extent as are the wings. While miniature in *obscura* resembles miniature in *melanogaster*, the use of the same name is not intended to signify that they are identical mutations.

Miniature is a recessive mutant of good viability and can be identified without difficulty. Its locus is between vermilion and reduced, which limits its usefulness since vermilion is more generally useful.

Locus of miniature

Miniature was used in a cross with vermilion and fused. Out of 224 individuals, 4 crossovers occurred between vermilion and miniature, and, in 3 of the 4, fused did not stay with vermilion. Miniature is placed, therefore, between vermilion and fused at a distance of 1.8 units from the former and 28.6 units from the latter. That this is the location of miniature is made more probable from results in a cross of miniature to white reduced. The results of this cross may be found in the table of summarized experiments. The position of miniature is clearly shown to be

about midway between white and reduced. Miniature and reduced have crossed over 8.6 percent of the time among 326 individuals. This amount of crossing over is less than that between vermilion and reduced. The experiment, which has just been referred to, shows in a striking fashion the poor viability of white. In every culture, each class containing white ran behind its reciprocal. More data are required before miniature can be accurately placed.

Singed (s_g)

A white male with abnormal bristles was found in culture 1312, October 15, 1920, during the second count of the bottle. This was the only male showing the new bristle characteristic, although three later observations were made on the culture, which produced, in all, 48 males. Since the new mutant, "singed," has shown only 6 percent of crossing over with white, singed probably arose by a mutation in the same X chromosome of the mother which carried the gene for white. The mother of 1412 was heterozygous for white and scutellar in opposite chromosomes. White had been received from her father. It is reasonable to conclude that the mutation to singed took place in the paternal X chromosome of the mother about the time of maturation, as it is certain that only a few gametes, at most, received the mutant gene, possibly only one.

Singed flies have the large bristles and the acrostichal hairs twisted and bent. The general effect is similar to that in the singed mutant of *D. melanogaster*. The females are sterile, as is the case with the more extreme allelomorph in the *melanogaster* singed. Singed is a recessive. Aside from the sterility of the females, which makes it impossible to keep a homozygous stock (entailing the necessity for selection in each generation), singed is a good mutant to work with. Its viability is fair. Its genetic usefulness is limited owing to its location close to vermilion. Whether singed is the same mutant as the similarly named mutant of *melanogaster*, or only a mimic cannot be determined from the present evidence.

Locus of singed

The approximate location of singed was determined by a cross of the original white singed male to a fused short female. The linkage relations were calculated from the males of six F_2 cultures (see table 17). The sequence which best fits the data, gives an order of white, singed, fused, short. The observed crossovers amount to 6 percent between white and singed, and 26 percent between singed and fused. These values are based

on 456 individuals. As the two values combined give a crossover value of 32 for white and fused, which is in good agreement with the value for the same region estimated from other crosses, we may assume that the conclusions arrived at as to the locus of singed are reliable.

A three-point cross, using singed against yellow vermilion, was made to determine with certainty on which side of vermilion singed had its locus. The males from four F_2 cultures are summarized in the table below. The absence of a double-crossover class gives the order yellow vermilion singed. Seven crossovers in 327 individuals give singed a position 2.2 units away from vermilion and very close to the probable locus of miniature.

TABLE 13

Nature of the cross: $\frac{\textit{yellow vermilion}}{\textit{singed}}$

REGION.....	0		1		2		TOTAL
Class.....	*y v*	s_g	*y* s_g	*v*	*y v* s_g	+	
	147	133	15	22	4	3	
Total.....	280		37		7		324

Polished (p_o)

The mutant polished was discovered in culture 1181, July 7, 1920. The female used in the culture was heterozygous for polished, which must have been in the opposite chromosome from yellow. It came, therefore, from the X chromosome of the father.

The polished males differ exceedingly in their appearance according to whether or not they are also slender. By itself, the gene for polished makes the body darker and shiny, and makes the bristles shorter and heavier. However, polished and slender together produce an unexpected result, as the bristles are made longer and are twisted in various ways. Polished is a recessive mutant. It would be more useful if it were located elsewhere in the chromosome than close to vermilion. With experience, polished can be identified accurately, though not rapidly, and can be used with all the other sex-linked mutants. Both sexes are fertile and the viability is fair.

Locus of polished

As polished appeared in a culture already heterozygous for yellow, it was apparent almost immediately that these two mutants were located

in the same region of the chromosome. Only two males out of 46 are recorded as crossovers, but it is probable that the classification at that time was not accurate, especially as yellow and polished are not a favorable combination of characters.

Polished was crossed to rough and showed a crossover value of 14 percent based on 140 flies. Bearing in mind that yellow and rough are about 34 units apart, the results then available indicated that polished was between yellow and rough, somewhere in the vicinity of vermilion. Polished was crossed to white reduced and shown to occupy a position between them. However, the exact position of the polished locus between vermilion and reduced must depend on data yet to be procured. The relative order of the three genes between vermilion and reduced, namely, singed, miniature and polished, is not yet ascertained with accuracy.

Dwarf (d_w)

Males, which were distinctly small in size, were observed in two sister cultures, 448 and 449, on December 23, 1919. These males were otherwise normal in structure. It was found that so much variation in size occurred that dwarf overlapped normal and made accurate classification impossible. For this reason the stock was not retained. The two mothers of cultures 448 and 449 were heterozygous for short as well as for dwarf, and in opposite chromosomes, as was shown by the fact that the dwarf flies were mostly not-short. The parents of the cultures that produced dwarf were taken from culture 291 where a short male was mated to a heterozygous short female. The mutation to dwarf must have occurred in the not-short X chromosome of that female and before maturation. The linkage of dwarf and short was determined roughly. Twenty-two crossovers were obtained in 218 males, or 10 percent of crossing over. Whether dwarf was to the right or left of short in the X chromosome was not determined.

Deformed (d_f)

Deformed was found in culture 257, November 3, 1919. The mother was apparently heterozygous for the gene, so that the mutation must have occurred in one of her parents. In deformed, the eyes do not have the regular curvature but are cone-shaped; the thorax is shortened; and the wings held out away from the body. The scutellum is flattened. Many of the vibrissae about the proboscis are absent. I am informed by Dr. A. H. STURTEVANT that this last condition is normal in certain other

species of Diptera. Only one sex comb is present on the front legs, as the tarsal joint which bears the second one is absent. These are the chief bodily effects produced by the gene, but there are many other lesser departures from normal which it would be difficult to describe. The deformed males did not breed, probably because they lacked enough vigor to mate. They were short-lived and succumbed to lack of food or to dryness far more readily than did normal individuals.

The location of deformed was not determined, as deformed is not the type of mutant which is useful in breeding or linkage experiments.

Extended (e^d)

The mutant extended was found in culture 953, April 15, 1920. The mother of this culture carried notch$_2$ in one chromosome and reduced rough in the other. The new gene was in the chromosome opposite notch, and more distant from notch than either reduced or rough. The mother received this reduced rough chromosome from her father, and the mutation must have occurred in it. On the rather meager data furnished by this culture (41 males), extended was placed about 26 units to the right of rough.

The mutant was named from the position in which the wings are held. They are spread away from the body, and curved downwards toward the ends. The mutant was not a useful one, as the individuals were weak and the males did not breed. It could have been kept only by breeding from heterozygous females, and was not retained.

Stubby (s_b)

Males with short stubby bristles were found by Dr. C. W. METZ in his culture L 553, December 11, 1919. Their main characteristic is the shortening of the macrochaetae and a bending or twisting of the bristles that occurs rather infrequently. It does not resemble the forked mutants of other species. This mutant was placed at my disposal by Dr. METZ. A cross of stubby to short showed that the two were closely linked, as only two crossovers were obtained in 150 individuals. Unfortunately, the further location of the gene for stubby could not be carried out as the strain was lost.

Lethal factors

Numerous sex-linked lethal factors have arisen by mutation during the investigation. Two of these have been discussed by the author (LANCE-

FIELD 1919) with reference to the nature of the sex ratio which resulted. The presence of a sex-linked lethal becomes evident, as only half the expected number of males is obtained. Such factors have been located in various regions of the chromosome map but are not sufficiently significant to merit attention here.

CONSTRUCTION OF THE CHROMOSOME MAP

The evidence showing that genes are arranged in linear series need not be presented here, as it has been adequately treated by STURTEVANT (1914, 1919), MULLER (1916), MORGAN (1919 a), BRIDGES (1919), and elsewhere. The chromosome map is one way of expressing a composite view of the linkage relations of the genes. The accuracy of the map depends on the statistical significance of the data from which the map is constructed. A brief statement of the essential points involved in the procedure is given by MORGAN and BRIDGES (1919).

It should be recognized that the amount of crossing over between the genes is not an absolute measure of their distance in the chromosome. There is probably no change in the distance relations of the genes which corresponds to the variations of linkage obtained by age change (BRIDGES 1916), or by temperature variations (PLOUGH 1918), or through presence of certain genetic factors (STURTEVANT 1919). It has been shown, in fact, that such linkage variations do not alter the sequence of the genes. There are some indications that the same relationship between chromosome distance and percent of crossing over does not hold in all regions of the same chromosome.

In making the map for the X chromosome of *D. obscura*, the gene for yellow has been taken as the zero point (although it is not a terminal gene in the linkage group) because the genetic length of the region between yellow and beaded cannot be determined until some suitably situated intermediate mutants are discovered. The region between yellow and fused, however, is filled with many genes so spaced that it is possible to work out the map distances with a fair degree of accuracy. Yellow is also used as the zero point to facilitate comparison of the sex-linked group of mutants with the sex-linked groups of other species of Drosophila. It is, however, still doubtful as to whether yellow should be considered an identical mutation to the yellow of other species.

The previous section of this paper has presented the evidence upon which the sequence of the mutant genes was based, and has indicated the method by which the various regions were oriented with respect to the others.

The loci of beaded, yellow, vermilion, fused, and short were early determined, and have been used as points of reference for the placing of other genes in their relative positions. The yellow-beaded distance is given as 42 units, which is based on the direct linkage value not corrected for a large amount of double crossing over, which undoubtedly occurs. Another long gap is shown between fused and ascute, and this again is based on the direct crossover value. Each of these regions will be considerably lengthened by using intermediate loci so that double crossovers can be detected. It was not thought profitable to attempt to calculate a correction based on the coincidence obtained in *D. melanogaster* until more evidence is secured that the coincidence relations are similar in the two species.

There is some difficulty in deciding what the distance between vermilion and fused should be. The sum of the intermediate distances is several units less than the distance based on direct crossing over. The first experiment, using vermilion and fused with no intermediate points, gave 30 percent of crossing over. However, the values obtained for vermilion reduced, reduced rough, and rough fused are: 8.4, 10.0, 1.2, which total less than 20. The discrepancy may be due partly to the use of values based on small numbers, as in the case of the rough-fused distance. The data for vermilion singed and singed fused are more extensive and give a total vermilion-fused distance of 28.2. The distance of 28 is adopted, which puts vermilion at 14 and fused at 42. Reduced is placed at 24 and rough at 36 until more extensive data are forthcoming.

In several cases the sequence is still in doubt. Such cases are compressed and fused; and the three genes between vermilion and reduced, namely, miniature, singed and polished. The order of the genes has been determined in most cases by crosses involving three or four loci in the same experiment.

The completed chromosome map is 170 units long. Crossovers are so frequent that three loci located at the two ends and in the center of the chromosome, respectively, show free crossing over with each other. This great length of the X chromosome in comparison with that of *D. melanogaster* and *D. virilis*, both cytologically and genetically, seems to be due to the addition of a considerable piece of chromosome, the source of which is, as yet, undetermined. The results indicate that in a general way the amount of crossing over in a chromosome is roughly correlated with its cytological length.

MUTANTS SIMILAR TO THOSE OF OTHER SPECIES AND CORRESPONDING CHROMOSOME REGIONS

It is not yet possible to decide just what region of the X chromosome of *D. obscura* corresponds to the X chromosome of *D. melanogaster* or

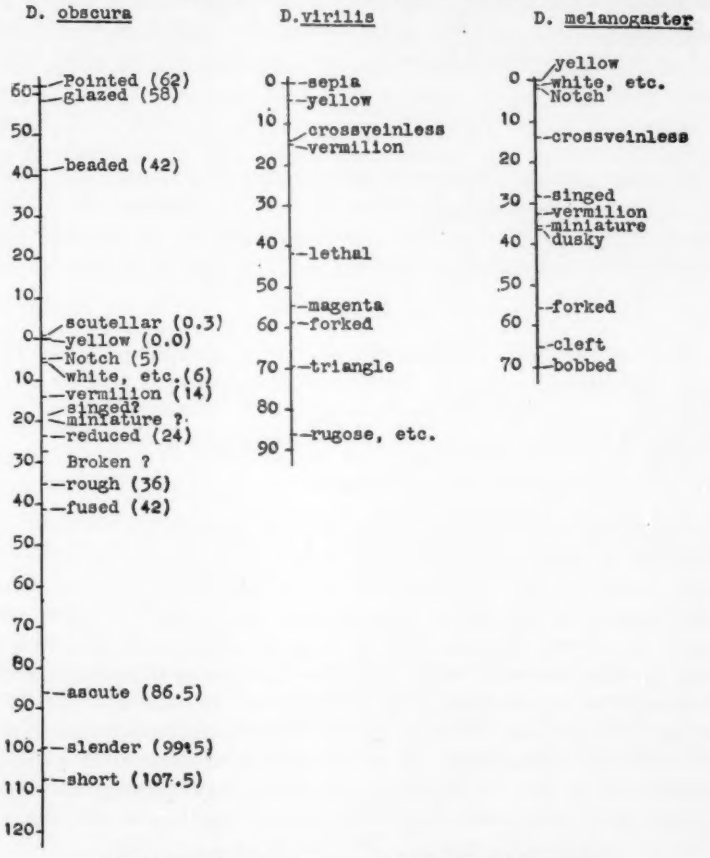

FIGURE 3.—Map of X chromosomes of 3 species of Drosophila.

D. virilis. Several sex-linked characters have been described which resemble in a striking manner those found in the sex-linked group of other species. However, the sequence, in the case of yellow, notch, white, is different in *melanogaster* and in *obscura*, and this difference makes any comparison uncertain.

The best method for determining that similar mutants in different species are identical, is to cross them and see whether they behave as allelomorphs. STURTEVANT (1921) crossed several sex-linked mutants of *D. melanogaster* and *D. simulans*, and showed that the mutant genes behaved as allelomorphs. Since interspecific crosses, using *obscura* and other species, have not been successful, this test cannot be applied here.

A brief comparison of mutants which resemble ones in other species may now be made.

Notch

Notch is a unique character in several respects and agrees very closely with the notch character in *D. melanogaster* and *D. funebris*. As STURTEVANT (1918) pointed out, when two mutants compared have several characteristics in common, the probability of identity is strengthened.

White and eosin

White and eosin are allelomorphs, as in *D. melanogaster*, and also show very close linkage to notch. The sexual dimorphism of eosin is similar in both species and the white-eosin compound is intermediate in eye color. A point of difference is the sterility of white females in *obscura*.

Yellow

This mutant resembles the yellow mutants of *virilis*, *melanogaster*, and *simulans*, of which the last two were shown to be actual allelomorphs by STURTEVANT (1921). The fact that the sequence of yellow, notch, and white eosin in *obscura* differs from the order of the similarly named characters in *melanogaster* makes the identity of yellow in *obscura* with the other yellows uncertain. The difference of sequence might be due to a rearrangement of that part of the chromosome, as BRIDGES (1917, 1919) suggested in his cases of duplication and transposition. If such a rearrangement has taken place, the characters may be identical with those of *melanogaster*, in spite of the change of sequence. At present, the case for identity seems to be weaker for yellow than for notch or white eosin.

Scutellar

Scutellar agrees with *melanogaster* scute in removing scutellar bristles and in showing very close linkage to yellow. In *melanogaster* no crossovers have been obtained, while in *obscura* 4 crossovers occurred among 1268 individuals. Scute has no post-vertical or ocellar bristles on the head. These bristles are present in the *obscura* mutant. The general similarity of

scute and scutellar and their linkage to yellow in each case may be only coincidence.

Vermilion

Vermilion is very similar in appearance to sex-linked vermilion in *virilis* and *melanogaster*, and also to autosomal scarlet in *obscura*, *melanogaster*, and *simulans*. In such a situation, similarity of appearance does not give very good evidence for genetic identity. The crossover value for yellow and vermilion is about 14 units in *D. virilis*, according to information furnished me by Dr. METZ, which agrees with the value found in *obscura*. The fact that the double-recessive eosin vermilion is nearly white in color in *D. melanogaster* and lemon in *D. obscura* suggests that the vermilion gene in *obscura* is not the same as the vermilion of *melanogaster*. The eosin vermilion map distances are 8 and 31, respectively, in the two species.

Singed

Singed agrees fairly well as regards its several peculiarities (including sterile females and its map distance from yellow) with *melanogaster* singed. In general, little reliance can be placed in bristle characters of this type for determining identity of mutant genes unless corroborative evidence is furnished by other closely linked characters, since both in *D. melanogaster* and in *D. willistoni* there is more than one sex-linked locus which is capable of producing mutations of this type.

Miniature

This character was named on account of its resemblance to *melanogaster* miniature, and it is an interesting coincidence that miniature in both cases is located just to the right of vermilion in the linkage group. Since the two vermilions do not seem to be the same, the identity of the miniatures is very doubtful.

Glazed

Glazed resembles the *virilis* glazed in the way the eyes are affected; and wax, an allelomorph of *virilis* glazed, has sterile females as does *obscura* glazed. In order to regard glazed as the same locus in the two species, it would be necessary to disregard the yellow vermilion comparison, which would orient the chromosomes in the other direction.

Ascute

Ascute is not similar to any sex-linked characters in other species, but it agrees very well in its effects with *melanogaster* ascute, with *obscura* grooveless, and with *virilis* hunch, all three of which are autosomal. There is no groove between thorax and scutellum, and a bit of brownish substance which looks like an excrescence is frequently at the junction of the thorax and scutellum. Ascute affords an example of how quite different genes may produce very similar and even unique characters.

Compressed

Compressed has several features characterizing it. Dr. METZ furnishes me the information that an autosomal character, telescoped, in *virilis*, has the same peculiarities. The number of features in common is almost as large as is the case between the various notch characters. The position of compressed in the chromosome map makes it improbable that the gene for compressed can be considered as identical with the gene for telescoped. This case shows that two similar characters, which may have as many as four features in common, are not necessarily to be regarded as identical, unless confirmatory evidence is obtained.

It is not now evident what part of the *obscura* X chromosome corresponds to that of either *melanogaster* or *virilis*. The different sequence for yellow, white, and notch, together with the occurrence of several characters which are similar to, but not identical or parallel with, ones in other species, has complicated the problem exceedingly. Sufficient data to solve it are not yet available.

I wish to thank Prof. T. H. MORGAN for valuable suggestions and assistance. Dr. A. H. STURTEVANT has helped me greatly with his advice and interest and I am further indebted to him for caring for my experiments at a time when I was unable to do so. Dr. C. W. METZ very kindly turned over to me his stocks of *Drosophila obscura* including several mutant races.

SUMMARY

1. A study of *Drosophila obscura* was undertaken in order to determine how the chromosome group of this species was related to that of *D. melanogaster* and of *D. virilis*.

2. In pedigreed cultures, 28 mutations have occurred in 23 loci in the X chromosome.

3. A consideration of the linkage relations of the various mutants indicates that the total amount of crossing over in the X chromosome of

D. obscura is greater than in any other linkage group heretofore studied. A map of the X chromosome based on these linkage relations shows a total distance of about 170 units, which exceeds the length of the maps of the second or third chromosomes in *D. melanogaster* by about 60 units.

4. There is evidence that certain of the mutants obtained are identical with sex-linked mutants in other species. The strongest cases are those of notch and of white-eosin. The loci of these genes in the *obscura* X chromosome are in the central region over 60 units from either end, while the loci of the similar mutants in *D. melanogaster* are very near the end of the X chromosome.

5. It is suggested that the greater length of the X chromosome, both cytologically and genetically, as compared with the X chromosomes of *D. virilis* and *D. melanogaster*, is due to the presence of a large piece of chromosome, attached to what corresponds to the left hand end of the *melanogaster* X chromosome.

6. Any attempt to identify the sex-linked characters of *D. obscura* with those of the two other species is difficult. Several mutants in *D. obscura* which are very similar in appearance to mutants in either *D. melanogaster* or *D. virilis* do not seem to be identical. The similarly named mutants, yellow, notch, and white-eosin, are not in the same linear order in *D. obscura* and in *D. melanogaster*.

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APPENDIX

TABLE 14

Sex-linked mutations of Drosophila obscura.

NAME	CHARACTER AFFECTED	SYMBOL	LOCUS	DATE	STOCK
Pointed.....	Wings	P	62	January, 1920	$Lethal_1$ test
Glazed.....	Eyes	g_z	58.	July 1920	s_c-y cross
Beaded.....	Wings	b	42.	August, 1919	s- (non-disjunction)
Scutellar.....	Bristles	s_c	0.3	April, 1920	s_l stock
Yellow.....	Body color	y	0.0	March, 1920	s_l stock
Notch.....	Wings, life bristles, etc.	N	5.	December, 1919	$Lethal_1$
White.....	Eye color	w	6.	February, 1920	Test of long
Eosin.....	Eye color	w^e	6.	August, 1920	s_c N linkage
Vermilion.....	Eye color	v	14.5	January, 1920	Test of s_b
Polished.....	Body color	p_o		July, 1920	y s_l linkage
Singed.....	Bristles	s_g		October, 1920	y w linkage
Miniature.....	Wings	m_n		July, 1920	y L_a linkage
Reduced.....	Bristles	r	24.	January, 1920	$Lethal_1$
Broken.....	Venation, life,	B_r		December, 1919	s- cross
Rough.....	Eyes	r_o	36.	January, 1920	r s linkage
Compressed.....	Thorax, eyes, etc.	c_o		March, 1920	b s linkage
Fused.....	Venation	f_u	42.	January, 1920	Inheritance B_r
Ascute.....	Thorax	a_s	86.5	August, 1920	s_c y v linkage
Slender.....	Bristles	s_l	99.5	February, 1920	Test sex ratio
Short.....	Venation	s	107.5	1916 (METZ)	Wild
Shorter.....	Venation	s^r	107.5	October, 1919	Test gap
Shortest.....	Venation	s^t	107.5	November, 1919	b s- (non-disjunction)
Dwarf.....	Size	d_w		December, 1919	s- cross
Extended.....	Wings	e_d		April, 1920	N r_o linkage
Deformed.....	Thorax, eyes, wings, etc.	d_f		November, 1919	Inheritance gap
Stubby.....	Bristles	s_b		December, 1919	No. 553 (METZ)
Lethals.....	Life		Various		Any stock

TABLE 15

Two-point linkage experiments.

LOCI	TYPE OF CROSS	NON-CROSSOVERS		CROSSOVERS		TOTAL
b N_2	*b* × *N*	0	201	118	0	319
b s_l	*b* × s_l	28	20	26	18	92
y N_2	*y* × *N*	0	129	10	0	139
y w	*y* × *w*	217	305	17	14	553
	y w × +	184	100	10	14	308
N_2 *w*	*N* × *w*	700	0	9	0	907
	N × *w*	240	246	4	1	491
N_2 w^e	*N* × w^e	340	374	4	0	718
N_2 s^r	*N* × s^r	109	0	98	0	207
b s	*b* × *s*	140	142	134	115	531
s_e *y*	s_e × *y*	142	109	0	1	252
s_e *v*	s_e × *v*	120	91	15	13	239
y v	*y* × *v*	59	50	9	3	121
p_o r_o	p_o × r_o	58	62	1	1	116
v p_o	*v* × p_o	70	44	10	10	140
v f_u	*v* f_u × +	46	32	11	20	109
v B_r	*v* × B_r	0	380	47	0	427
c_o f_u	c_o × f_u	208	140	23	2	373
N v	*N* × *v*	131	0	0	8	139
B_r f_u	B_r × f_u	0	106	22	0	128
B_r *s*	B_r × *s*	0	28	29	0	57
s d_w	*s* × d_w	42	61	7	4	114
s s_b	*s* s_b × +	77	71	2	0	150
s s_l	*s* × s_l	130	147	13	12	302

NOTE. In these tables the same arrangement has been followed as that used by STURTEVANT (1921). "The column headed 'Loci' shows not only the loci concerned but also their sequence. In the third and following columns classes are entered under their headings indicating the type of crossing over they represent. In every case that class which includes the individuals bearing the wild-type allelomorphs in the left-hand locus concerned is placed first, and is followed by the contrary class."

TABLE 16

Three-point linkage experiments.

LOCI	TYPE OF CROSS	NON-CROSSOVERS		SINGLE CROSSOVERS				DOUBLE CROSSOVERS		TOTAL
				Region 1		Region 2				
P g_s *v*	*P* × g_s *v*	40	41	2	1	40	41	0	4	176
P b v	*P* × *b v*	40	52	21	0	32	52	0	7	204
P y a_2	*P* × *y* a_2	18	68	42	5	23	38	37	14	245
g_s *b y*	g_s *y* × *b*	18	10	2	2	9	11	1	1	54
g_s *b v*	g_s × *b v*	25	18	10	11	5	9	0	0	78
b w s	*b s* × *w*	121	157	95	77	91	143	103	50	837
b v s	*b s* × *v*	21	18	18	16	26	34	21	16	170
b N s	*b s* × *N*	0	46	0	23	0	44	34	0	147
b r s	*b s* × *r*	32	48	33	18	30	21	34	25	241
b f_u *s*	*b* f_u *s* × +	62	45	32	47	49	31	38	47	351
s_c *y* w^e	s_c *y* × w^e	141	166	12	3	0	2	0	0	324
s_c *y v*	s_c × *y v*	109	92	0	0	27	28	0	0	256
	s_c *v* × *y*	74	69	0	0	10	11	0	0	164
s_c *y* r_0	s_c *y* × r_0	41	44	0	1	10	5	0	0	101
s_c r_0 sl	s_c sl × r_0	33	27	9	9	20	27	14	8	147
y N_2 w^e	*y N* × w^e	167	201	11	7	4	1	0	0	391
	y N × w^e	0	569	0	22	8	0	0	0	599
	y w^e × *N*	214	175	9	10	1	5	0	0	424
	y w^e × *N*	0	152	14	0	0	1	0	0	167
y N v	*y v* × *N*	0	162	15	0	0	11	0	0	188
y v s_9	*y v* × s_9	133	147	22	15	3	4	0	0	324
N_2 *r* r_0	*N* × *r* r_0	0	71	29	0	11	0	0	2	113
w p_0 *r*	*w r* × p_0	87	49	2	1	4	4	0	0	147
w m_n *r*	*w r* × m_n	216	62	16	4	23	2	2	1	326
v B_r r_0	*v* r_0 × B_r	0	77	0	7	5	0	0	0	89
v r r_0	*v* × *r* r_0	91	224	16	7	10	20	3	5	376
c_0 a_2 sl	c_0 a_2 × sl	80	49	77	47	11	13	6	8	291
c_0 a_2 *s*	c_0 × a_2 *s*	91	95	98	50	27	26	28	13	428

TABLE 17

Four-point linkage experiments.

LOCI	NATURE OF CROSS	NON-CROSS-OVERS		SINGLE CROSSOVERS						DOUBLE CROSSOVERS						TRIPLE		TOTALS
				1		2		3		1:2		1:3		2:3		1:2:3		
g_s *y v s*	g_s *y v s* × +	38	33	21	21	11	3	29	22	2	7	26	26	4	5	4	5	257
g_s *y* f_u *s*	g_s *y s* × f_u	6	11	8	3	4	2	10	6	0	2	9	2	5	2	1	3	74
g_s *b y v*	g_s *y* × *b v*	33	16	9	6	16	16	2	1	0	1	0	3	4	3	0	0	110
b v m_n f_u	*b v* f_u × m_n	65	32	22	38	2	1	8	34	0	0	16	5	0	0	0	1	224
b v f_u *s*	*b* f_u *s* × *v*	40	33	20	22	9	29	40	33	15	8	26	24	15	17	9	1	341
b v B_r f_u	*b v* f_u × B_r	0	29	15	0	1	0	0	12	0	3	0	17	3	0	0	1	81
b c_0 f_u *s*	*b* c_0 × f_u *s*	46	37	39	47	3	0	56	40	1	3	24	31	2	0	0	1	330
v r r_0 f_u	*v* f_u × *r* r_0	88	120	18	2	6	13	0	2	2	1	0	0	0	1	0	0	253
w s_9 f_u *s*	*w* s_9 × f_u *s*	101	65	8	0	55	11	96	53	2	3	10	4	38	7	1	2	456
c_0 a_2 sl *s*	c_0 a_2 × sl *s*	87	29	71	41	12	0	7	1	4	7	0	2	0	0	0	0	261

TABLE 18

Summarized data for each pair of loci in the X chromosome.*

REGION	TOTAL	CROSSOVERS	PERCENTAGE OF CROSSING OVER
y N_2	1712	88	5.2
y w	1185	70	5.9
y v	1231	179	14.5
y r_0	212	39	18.4
y s_c	1268	4	0.3
y b	275	115	41.8
N_2 *w* or w^c	3419	37	1.1
N_2 *v*	327	19	5.8
N_2 *r*	113	31	27.4
N b	466	175	37.5
w s_g	456	30	6.8
w s	837	387	46.2
w b	1112	432	38.8
v m_s	224	4	1.8
v p_0	116	2	1.7
v s_g	324	7	2.2
v r	668	56	8.4
v B_r	508	55	10.8
v f_u	341	103	30.2
m_s *r*	326	28	8.6
p_0 *r*	147	8	5.4
p_0 r_0	140	20	14.3
s_g f_u	456	119	26.0
r r_0	742	74	10.0
r f_u	275	40	14.5
B_r r_0	89	7	7.8
r_0 f_u	253	3	1.2
c_0 f_u	703	35	5.0
c_0 a_s	428	189	44.1
a_s s_l	552	63	11.4
f_u *s*	1753	826	47.2
a_s *s*	428	94	21.9
s s_l	302	25	8.2
s s_b	150	2	1.3
s b	531	249	46.9
s_c *v*	239	28	11.7
b g_s	242	39	16.1
b P	204	28	27.4
g_s *p*	176	7	4.0

*NOTE. The data used in the above table are comprised entirely of data in which no intermediate locus was present between the loci concerned. For instance, in a three-point cross, the direct crossover values can be calculated for the first and second, and for the second and third loci; then by ignoring the middle locus, the direct crossover can be calculated for the first and third loci from the same experiment. This third value is not included in the above table.

THE INHERITANCE OF SKI WINGS IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

While a great number of the mutant characters of *Drosophila melanogaster* have been found to follow a simple mode of Mendelian inheritance, not a few involve more or less complex factor relations. These latter have been of great value in demonstrating the generality of the phenomena involved in Drosophila studies. To those who have worked much with Drosophila the simplicity and definiteness of the results is regarded as a natural consequence of the material which has been used in the studies. The original wild type of the species occurs in nature over a very wide area, yet it is apparently monotypic in form and possesses a germinal constitution essentially identical throughout its extensive distribution.

From this relatively invariable wild type, mutations have been discovered from time to time which have evidently involved changes in specific elements in the germinal material. Since these changes have occurred either directly from the wild type or in other mutant stocks not far removed in derivation from it, they have evidently been of a kind eminently suited for genetic investigation. Of all plant and animal material, that obtained from Drosophila has most nearly approached the ideal for genetic experimentation, for it has furnished a wide array of forms which differ from a single wild type only in the simple characters which they exhibit. One need only compare this condition with that which obtains in most plants and animals, where investigations have been conducted with varieties which have long been established, and which almost invariably differ from one another in a large number of characters, and even more complexly in their germinal make-up, to realize, aside from its ease of culture and short life cycle, the unique superiority of this material for genetic research. Even in some of the more involved cases, Drosophila investigations have been relatively simple as compared with the complexity which is often exhibited in other forms. On the other hand, such typical investigations as those of ALTENBURG and MULLER (1920) on truncate wings, BRIDGES (1919) on modifiers of eosin eye color, MULLER (1918) on balanced lethals, BRIDGES and MOHR (1919) on vortex, STURTEVANT (1918) on number of spines in dichaete strains, MORGAN (1919) on notch wings, and the epoch-making investigations of BRIDGES (1916, 1917, 1919) on non-disjunction, deficiency, duplication and the like, reveal how far our conceptions of germinal structure and behavior have been determined by the results of the more complex Drosophila investigations.

Now although several investigations have been made with characters which are the result of complex factor interactions, the results of such studies have not often been as clean-cut as those from other characters, because of the variability of the forms in question. Thus, BRIDGES and MOHR (1919) point out in their analysis of vortex that while the character depends essentially upon the interaction of two genes, $v_{o\text{II}}$ and $V_{o\text{III}}$, males usually exhibit vortex only when of the composition $\frac{v_{o\text{II}}}{v_{o\text{II}}}\ \frac{V_{o\text{III}}}{V_{o\text{III}}}$, but among the females approximately 20 percent of those of the constitution $\frac{v_{o\text{II}}}{v_{o\text{II}}}\ \underline{V_{o\text{III}}}$ also show a variable development of the vortex character. In addition to these complications a special relation of the character to streak was discovered; and two additional modifying genes were demon-

strated which had specific effects upon its development. In most plants and animals such a complex series of interactions would be extremely difficult of precise analysis, but in Drosophila the development of a special technique makes it possible to eliminate guesswork, and to follow genes from generation to generation with perfect assurance, even though their effects are for the time being completely suppressed. Nevertheless it would seem probable that the precise analysis of other more definitely stable characters involving complex factor relations would add to the general acceptability of the results.

In the present paper the results of the investigation of a bigenic character which does not exhibit phenotypic difficulties are reported. The analysis has been carried out more completely than was necessary for a determination of the actual factor relations at work in the production of the character, because the absence of phenotypic variability in it made it possible to demonstrate the adequacy of the bigenic hypothesis in a variety of ways not possible had such variability occurred. Moreover, it is recognized that there are those who would wish to see not only the consequences of the analysis pointed out; but would also desire a full demonstration of actual experimental results in confirmation of the analysis. We have attempted, therefore, to devise critical experiments under control which would satisfy this very natural demand.

ORIGIN AND HISTORY OF SKI

The history of the ski stock runs back to October 10, 1915. On that date a quart fruit-jar containing a liberal supply of fermenting banana was exposed in the Botanic Garden of the UNIVERSITY OF CALIFORNIA in order to secure wild strains and species of Drosophila. One week later the culture was brought into the laboratory. On October 20th the banana in this jar, together with the contained larvae, was transferred to a pint milk bottle. On October 28th the culture was examined and found to contain 28 *melanogaster* and 6 *obscura* individuals. Of the 28 *melanogaster*, 4 were white-eyed males. At the time of examination it was noted that these white-eyed flies were probably the result of contamination from laboratory sources after the fruit jar had been brought into the laboratory.

Nevertheless these four white-eyed males were saved and mated in mass culture to wild-type females from a laboratory stock. Culture Cr10, thus established, produced 45 wild-type flies. From these wild-type flies two further mass cultures were raised, Cr49 and Cr50, each from five pairs of flies (table 1). The large number of ski flies secured from

these cultures indicates that ski appeared originally as a segregation product. Inasmuch as the white-eyed males which were mated to females from a laboratory stock of wild-type flies were doubtless escapes from our laboratory stock of white, there is every reason to believe that ski did not derive its origin from the fresh wild stock secured in the exposed fruit jar.

A stock culture of ski was eventually established from culture Cr49, and this stock was maintained in the ordinary routine fashion. It was observed, however, that matings of ski flies often produced some wild-type individuals, but this fact was ascribed to phenotypic variation in the character. It was also observed that in stock cultures of ski it was necessary to select for ski in order to prevent "reversion" to wild-type.

TABLE 1

Original cultures from which ski flies were secured.

	WILD-TYPE		WHITE		SKI		WHITE SKI	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
Cr49.....	316	164	..	142	18	4	..	5
Cr50*.....	146	76	6	7	1	1	..	..

* The 6 white females were produced after the culture had been yielding flies for over a month, consequently they clearly belonged to a second generation produced in the same bottle.

Since ski closely resembled descriptions of the jaunty and curled mutants, which had been discovered at COLUMBIA UNIVERSITY, we obtained stocks of these flies from Professor MORGAN. Reciprocal matings of curled × ski and jaunty × ski made in June, 1916, in all cases gave wild-type F_1, thus demonstrating that ski was a distinct new character.

Up to 1919 a number of attempts were made to determine the mode of inheritance of ski, but these came to nothing on account of the failure to recognize its bigenic nature. The experiments were all carried out under the assumption that ski, like jaunty and curled, which it resembles phenotypically, was a monogenic character. The appearance of wild-type flies in cultures which should have been all ski was ascribed to phenotypic variation analogous to that which had already been reported for certain characters such as club wings. A rather extensive series of investigations designed to determine the locus of ski also gave discordant results, due to the fact that ski flies always segregated out in a much lower ratio than that expected for a simple recessive character. During this period proper stocks were not on hand in the Laboratory of Genetics of the UNIVERSITY

OF CALIFORNIA, to carry out expeditiously a precise analysis of a complex character. In 1919, however, the authors secured additional stocks of flies from COLUMBIA UNIVERSITY, and embarked upon an extensive series of experiments designed to demonstrate its mode of inheritance.

DESCRIPTION OF SKI

As stated before, ski bears a close resemblance to the jaunty and curled mutants discovered at COLUMBIA UNIVERSITY. It is in fact the form which BRIDGES and MORGAN (1919) call "California curled" in their discussion of jaunty. There is also a sex-linked jaunty, now extinct, which belongs to the same "genus of mutants." Ski is variable in expression, but if the wings are fully developed, there is never the slightest difficulty in separating it quickly and accurately from normal-winged flies. In its less extreme expression, simplex ski, it is almost identical with jaunty, and cannot be separated from it in mixed cultures. In this expression the wings are slightly, but distinctly, bent upward at the tips, the curvature usually involving only the terminal half of the wing, although some individuals do show a rather uniform upward sweep of the wings from the basal portion. The more extreme expression, duplex ski, is typically characterized by a marked curvature of the wings closely approximating curled in appearance. In this expression the wings are often so strongly recurved that the tips point directly forward. Although these two types of ski are fairly distinct, the variation in expression among them is such as to render accurate and rapid classification of mixed populations into the two ski classes out of the question. The genetic significance of the two classes will be pointed out below.

Although it would be impossible to separate ski from jaunty and curled in mixed populations with the unaided vision, there are certain associated characteristics which are revealed by magnification with a binocular which distinguish it from other, like forms. Thus in jaunty the wings are slightly thicker in texture than those of normal flies, and they are frequently corrugated transversely. In ski the texture does not seem to differ appreciably from normal, and transverse corrugations seldom occur. In curled the wings are slightly thinner than normal and have a waxy or silvery texture. Often the upper surface of the wing has a crumpled appearance very much like that of a thin piece of paper unevenly bent. Sometimes this crumpling results in a blistered appearance of the tissue of the wing. The wings of jaunty are often more strongly curved on the inner side, and they may have a slight outward slant. The outward slant is even more noticeable in ski, and it is still more striking in curled.

In jaunty and curled the wings are almost flat transversely and evenly upturned, but in curled the upper surface is somewhat convex, which may account for the crumpling of the wing tissue in this form. In curled the wings are noticeably indented on the inner margin at the end of the fifth longitudinal vein, whilst in jaunty and ski, the margin is evenly curved at this point.

The body color of jaunty is said to be slightly darker than that of wild-type flies. In our experience with jaunty strains isolated from a multiple stock, this difference, if present at all, is extremely slight. Ski flies do not show any detectable difference in body color from wild-type. The stocks of curled which we have had, however, have all exhibited a very marked dark body color, almost as dark as that of black flies. Whether this character is associated with curled has not been determined; but if it is, it will provide a ready means for identifying curled.[1] In jaunty, the posterior scutellars point straight backward as they do in wild-type, but in ski and curled these bristles point inward and laterally, crossing near the middle.

The most noticeable positive associated characteristic of ski is exhibited by the costa or marginal vein of the wing. In wild-type this vein has an even, outward curve in prolongation of the first longitudinal vein. In typical ski, however, the costa exhibits a sharp infolding, making a deep "crimp" in the proximal portion of the marginal cell. This "crimp" is merely a partial persistence of the deep fold which occurs in this region of the wing pad of newly-emerged flies. It is an upward and inward fold in the wing, and apparently is more pronounced the more strongly recurved the wing. In simplex ski the "crimp" is often very slight, but still detectable. It is not present in jaunty or curled flies, consequently it is possible to make a rough separation of a mixed population on the basis of the presence or absence of this "crimp" in the wings. Along with this "crimp" there is more or less "buckling" all along the length of the costa, and this is also more pronounced in the more strongly recurved wings. The tissue of the marginal cell is crumpled somewhat as a consequence of the irregularity of the costa.

Although there is a certain amount of variation in the expression of ski, it is never of such a nature as to confuse separation of ski from wild-type. The least-marked expression of ski is perfectly definite and readily separable from normal without recourse to magnification. Its ready and unmistakable separation from wild-type makes it a very favorable character for investigation.

[1] BRIDGES reports that this dark color is due to the same gene that produces curled, or, less probably, to a very closely linked modifier.

BIGENIC NATURE OF SKI

During 1918 at a time when it seemed possible that we would be unable to perpetuate ski ourselves or to do further work with it, a culture of it was sent to the Zoölogical Laboratory of COLUMBIA UNIVERSITY. In 1919 when we resumed work with it it soon became evident that we would need appropriate stocks of dominant characters in order to analyze it expeditiously. Accordingly we wrote to Professor MORGAN for cultures of the dominant mutants, bar, star, and dichaete. In our letter we called attention to our work to date, which showed that with ski and wild-type segregants from ski the following series of results had been secured:

Wild-type × wild-type gave uniformly wild-type flies only.
Ski × ski gave either all ski or 3 ski : 1 wild-type.
Ski × wild-type gave either all ski or 1 ski : 1 wild-type.

We also pointed out that by continued sister × brother matings we had been able to establish a stock breeding true for ski. This statement, however, later proved to be incorrect, for the stock subsequently produced wild-type flies. In reply Doctor STURTEVANT, to whom Professor MORGAN referred this letter, stated that he and Doctor BRIDGES had also performed some preliminary experiments with ski. They were able to confirm our results and in addition to point out that F_1 star dichaete males from matings of star dichaete × ski back-crossed to wild-type segregants from ski gave two types of results, namely,

(a) 1 star dichaete : 1 star : 1 dichaete : 1 ski
(b) 1 star dichaete : 1 star : 1 dichaete : 1 wild-type

On the basis of these results they outlined a hypothesis, which we shall refer to as the bigenic hypothesis, to the effect that ski depends upon a difference from wild-type in two genes, one a dominant gene located in the second group, and the other a recessive located in the third. To the second-chromosome gene we assign the symbol, $S_{i_{II}}$, and to the third, $s_{i_{III}}$, and throughout this paper we shall use the abbreviations, S_i and s_i, respectively, for these symbols. Neither of these genes alone produces any difference from wild-type. For the production of ski it is necessary that s_i be homozygous, and that S_i be either in the heterozygous or homozygous condition. STURTEVANT and BRIDGES recognized the occurrence of $\frac{S_i}{S_i}\ \frac{s_i}{s_i}$ flies, for in this case S_i, although dominant, exhibits no recessive lethal effect such as is shown by most of the dominant mutants in Drosophila.

Since we have found the bigenic hypothesis fulfilled at all points, it seems well in the interest of clarity of presentation to reverse the customary procedure, and to present the general results of the analysis before giving the experimental data. We have accordingly constructed table 2, which gives a convenient summary of all the ratios of wild-type : ski which may be secured from different matings involving the genes, S_i and s_i and their normal allelomorphs. We have summarized the results of these forty-five different types of reciprocal matings below by grouping those which give the same ratio of wild-type : ski together and stating the ratios in terms of 16 for the sake of more ready comparison:

27 give 16 wild-type : 0 ski
1 gives 14 wild-type : 2 ski
1 gives 13 wild-type : 3 ski
5 give 12 wild-type : 4 ski
1 gives 10 wild-type : 6 ski
6 give 8 wild-type : 8 ski
1 gives 4 wild-type : 12 ski
3 give 0 wild-type : 16 ski

Such a variety of ratios would be very puzzling in any bisexual form in which the methods of analysis had not been developed to the perfection found in Drosophila. In Drosophila, however, it is possible to test this entire array of matings under perfect control.

TABLE 2

The theoretical results of the 45 possible types of reciprocal matings involving the genes S_i and s_i and their normal allelomorphs. The genotypes of the parents are given along the top and the phenotypes in the left-hand column. The genotype of each phenotype may be obtained by reference to the genotype of the same number. The ratios are stated throughout in terms of wild-type : ski.

PHENOTYPES	TYPE NUMBERS AND GENOTYPES								
	1	2	3	4	5	6	7	8	9
	– –	$\frac{S_i}{}$ –	$\frac{S_i}{S_i}$ –	– $\frac{s_i}{}$	– $\frac{s_i}{s_i}$	$\frac{S_i}{}$ $\frac{s_i}{}$	$\frac{S_i}{}$ $\frac{s_i}{s_i}$	$\frac{S_i}{S_i}$ $\frac{s_i}{}$	$\frac{S_i}{S_i}$ $\frac{s_i}{s_i}$
9 duplex ski	1 : 0	1 : 0	1 : 0	1 : 1	0 : 1	1 : 1	0 : 1	1 : 1	0 : 1
8 wild-type	1 : 0	1 : 0	1 : 0	3 : 1	1 : 1	3 : 1	1 : 1	3 : 1	
7 simplex ski	1 : 0	1 : 0	1 : 0	3 : 1	1 : 1	5 : 3	1 : 3		
6 wild-type	1 : 0	1 : 0	1 : 0	7 : 1	3 : 1	13 : 3			
5 wild-type	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0				
4 wild-type	1 : 0	1 : 0	1 : 0	1 : 0					
3 wild-type	1 : 0	1 : 0	1 : 0						
2 wild-type	1 : 0	1 : 0							
1 wild-type	1 : 0								

Some of the consequences of this hypothesis are very curious. Thus, duplex ski, $\frac{S_i}{S_i}\ \frac{s_i}{s_i}$, mated to wild-type, $-\ \frac{s_i}{s_i}$, gives F_1 ski, $\frac{S_i}{-}\ \frac{s_i}{s_i}$, and F_2, 3 ski : 1 wild-type. Here ski behaves as a simple dominant to wild-type, the wild-type segregants obtained in F_2 breed true when mated *inter se*, and of the ski individuals one-third are homozygous for ski when tested against this wild-type race and the remaining two-thirds are heterozygous. However, when individuals of this same duplex ski race are mated to members of a different wild-type race, $\frac{S_i}{S_i}\ -$, F_1 is wild-type, $\frac{S_i}{S_i}\ \frac{s_i}{-}$, and F_2 consists of 3 wild-type : 1 ski. In this latter case the F_2 ski segregants breed true when mated *inter se*, but of the wild-type segregants, one-third are homozygous for wild-type and the other two-thirds are heterozygous for ski. In this case wild-type behaves as a simple dominant to ski, the exact reverse of the behavior described in the first cross.

There are curious linkage relations which also depend upon the bigenic nature of ski. Thus sepia, $-\ \frac{s_e}{s_e}$, mated with duplex ski, $\frac{S_i}{S_i}\ \frac{s_i}{s_i}$, gives wild type F_1, $\frac{S_i}{-}\ \frac{s_i}{s_e}$, and in F_2 9 wild-type : 3 ski : 4 sepia : 0 sepia ski. This ratio does not differ strikingly enough from the 2 : 1 : 1 : 0 ratio normally expected from a mating between two simple recessive mutants whose genes lie in the same group, to be detectable with certainty under ordinary conditions. It would, therefore, unquestionably be interpreted as a ratio indicating that ski is a simple recessive character dependent upon a gene located in the third group. But if we should take a sepia race of the constitution, $-\ \frac{s_e\ s_i}{s_e\ s_i}$, which would not differ in appearance from the one used in the first cross, our F_1 would be ski, $\frac{S_i}{-}\ \frac{s_e\ s_i}{s_i}$, and F_2 would consist of 9 ski : 3 wild-type : 3 sepia ski : 1 sepia, indicating with equal certainty that ski is not a member of the third group. It would not profit us much to follow out this analysis more completely at this point. We have accomplished our purpose, if we have laid a foundation upon which the results of the experiments may be discussed expeditiously.

The duplex ski flies referred to in the description of ski are those of the genetic constitution, $\frac{S_i}{S_i}\ \frac{s_i}{s_i}$, while simplex ski flies are those of the hetero-

zygous genotype, $\frac{S_i}{\quad}\ \frac{s_i}{s_i}$. The term ski is used when the ski class consists of both duplex and simplex flies, in other cases the appropriate term, duplex or simplex, is used. A race of the constitution, $\frac{\quad}{\quad}\ \frac{s_i}{s_i}$, indistinguishable in appearance from ordinary wild-type, we call an s_i-wild-type race; one of the constitution, $\frac{S_i}{S_i}\ \frac{\quad}{\quad}$, also indistinguishable from ordinary wild-type, we call an S_i-wild-type race.

EXPERIMENTAL METHODS

The experimental methods employed in Drosophila studies are doubtless familiar enough to most investigators to make a detailed account of them unnecessary in this paper. Since some deviations from the practice employed at COLUMBIA UNIVERSITY were, however, necessary on account of the equipment on hand, we have thought it desirable to describe briefly the particular methods which we have used. It has been found necessary to give careful attention to the conditions of experimentation in order to secure satisfactory results with ski. It should be distinctly understood that these methods are not recommended in preference to those which have been outlined in detail by BRIDGES (1921), for a small amount of comparative work which we have done indicates that somewhat better results may be secured by those methods and that less time is necessary for the care and examination of cultures.

General culture methods

Standard wide-mouthed homeopathic vials of 8-dram capacity (equivalent to 29.6 cc) were used for culture bottles. The vials were prepared for use by inserting a strip of towel paper and a cotton plug in them, and they were then thoroughly sterilized in a hot-air sterilizer. Fermenting banana was used for food. Ripe bananas were cut into sections 0.5 to 1.0 cm thick, covered with water in a fruit jar, and steam-sterilized for about fifteen minutes. After cooling, a small quantity of Fleischmann's yeast was added to the jar, and then fermentation was allowed to go on at room temperature for about twenty-four hours before using the material. As cultures were needed they were prepared by simply inserting about 5 cc of fermenting banana into each vial. This amount of banana was found sufficient under proper conditions to supply food for as many as 200 flies from the time of hatching through their entire life-cycle. Cul-

tures were kept in a small incubator room maintained at a constant temperature of 25°C by means of a thermostatically-controlled electric heating unit.

The use of small vials instead of the larger half-pint milk bottles has been found to give fairly satisfactory results. Naturally fewer flies can be grown in such vials, so that in order to raise large progenies from a single mating, it is necessary to transfer the parents to successive vials at short intervals. When this procedure is followed, the full progeny of a pair of flies may be secured. In one case a pair of flies which was transferred successively at five-day intervals yielded 919 flies in eight sub-cultures. The method of frequent sub-culturing increases the amount of time and attention necessary for the work, but it has some compensations in the greater ease of examination of cultures.

Maintaining stocks

In Drosophila investigation, it is usually necessary to synthesize and maintain a certain number of stock strains. In our work these stocks were studied and examined until they were known to be established for their characters, after which they were merely examined occasionally in order to determine whether they were continuing true to type. Stocks were continued merely by separating out two pairs of flies from an old culture for the establishment of a new one. New cultures thus established were ordinarily sub-cultured at four or five day intervals. By this method an adequate supply of any stock was always on hand, and danger of losing weak stocks or those possessing low viability was reduced to a minimum.

Procedure with experimental cultures

Cultures upon which counts were made were always established with a single pair of flies. Virgin females were isolated from vials which had previously been emptied of all flies. The individual pedigree of every culture was known absolutely. The parents of experimental cultures were transferred five successive times at two-day intervals to new sub-cultures. Two-day sub-cultures rarely produce more than 100 flies each, so that the amount of food present is adequate for the development of all the larvae. Larval competition seemed to be of no serious consequence, as the results will show, when sufficient food for all was present in the vial; but when the food supply was insufficient, or the food became dry, serious distortion of ratios would often occur through partial elimination of weak classes or those having a slightly longer period of larval development. That the

food supply actually was sometimes inadequate, particularly in cultures which had given an unusually high yield, was shown by the emergence of pale, under-sized flies towards the end of the period of examination.

Examination of flies

Cultures were examined every second day during the period of emergence. Flies were etherized in the usual manner for examination. After the flies had been separated into the appropriate classes counts were recorded on form blanks conveniently devised for the purpose, one blank being used for each sub-culture. In case of classification for microscopic characters, such as star and spineless, a binocular was used. It was necessary to exercise care throughout to retain cultures until a full count had been secured, otherwise the proportion of ski flies would be low.

CHARACTERS EMPLOYED IN THE ANALYSIS

To avoid complication the number of stocks employed in the analysis was kept at a minimum. For the sake of completeness and ready reference they are enumerated here with brief descriptions of them. They were all obtained from the Zoölogical Laboratory of COLUMBIA UNIVERSITY, so that identity with their stocks is assured.

Star, gene *S*, locus II–0.0, is a dominant mutant characterized by an irregular jumbling of the facets of the eye. It is lethal when homozygous. It requires proper illumination and magnification for separation from wild-type, and care must be exercised to avoid confusing it with other like malformations of the eyes. A full account of this form, together with an illustration, is given by BRIDGES and MORGAN (1919).

Black, gene *b*, locus II–46.5, is an incompletely recessive mutant having a greenish-black coloration of the body and wings. Wild-type and heterozygous flies are treated as one class, readily and accurately separable from black without magnification. Its locus is the base of reference for the entire second chromosome. It is illustrated in color and fully described by BRIDGES and MORGAN (1919).

Sepia, gene s_e, locus III–25.8, is a recessive form characterized by a distinct dark-brownish coloration of the eyes. It is also readily separable from wild-type flies without magnification. Although it has long been known, no complete account of it has yet been published.[2]

Dichaete, gene *D*, locus III–38.5, is a dominant mutant with outwardly spread wings and a reduced number of dorso-central and scutellar bristles. Like star, it is lethal when homozygous. It is readily separable without

[2] The locations of the third-chromosome mutants are taken from a map published by BRIDGES (1921).

magnification by means of the wing character. For examination of reduction in number of bristles magnification is, of course, necessary. Dichaete has been described and illustrated by STURTEVANT (1918).

Spineless, gene s_s, locus III–54.2, is a recessive mutant which exhibits extreme reduction in the size of all body spines. The authors have always used magnification in separating it, although it is possible for a skilful investigator to classify it with the unaided vision. Spineless has been used in a large number of investigations, but no thoroughgoing account of it has yet been published.

None of these characters interferes with any other one or with ski to such an extent as to preclude the use of any combinations involving it. Thus, dichaete and ski produce a fly, dichaete ski, which exhibits a combination of the two characters. The wings are spread out from the body as in dichaete, but instead of being straight or slightly curved downward, they are strongly recurved at the tips. Similarly dichaete and spineless do not conflict, for if all the dorso-central and scutellar spines should be absent on account of the action of dichaete, it would still be possible to recognize dichaete spineless flies by the effect of spineless on the other spines of the integument. There are, therefore, no special difficulties involved in the handling of these characters in the same experimental cultures aside from those which have been mentioned for each character.

EXPERIMENTAL PROOF OF THE BIGENIC HYPOTHESIS

The most convenient method of determining the group to which a new gene belongs is that developed by BRIDGES and MORGAN (1919) in which the dominant characters star and dichaete are used as markers of the second and third groups, respectively. For example, if sepia be crossed with star dichaete, F_1 consists of four classes, namely,

1 star dichaete : 1 star : 1 dichaete : 1 wild-type.....(1)

If we now back-cross F_1 star dichaete males, there being no crossing over in them, to sepia females, we obtain the following segregation ratio:

1 star dichaete : 1 star sepia : 1 dichaete : 1 sepia.....(2)

Since sepia does not assort independently of dichaete, it must belong to the third group. If we perform the same series of experiments with black, the back-cross gives the following distribution:

1 star dichaete : 1 star : 1 black dichaete : 1 black.....(3)

Here black does not assort independently of star, and it must, therefore,

belong to the second group. In case the character belongs to neither the second nor the third group, it would assort independently both of star and of dichaete, and it would then be a question of the first or fourth group. The sex distribution would indicate whether or not it belonged to the first group. If the character showed no sex linkage, it would be assigned by exclusion to the fourth group. Should the character in question prove dominant, appropriate modifications in the matings may be made for the determination of the group.

When duplex ski is crossed with star dichaete, F_1 conforms to ratio (1) above. When F_1 star dichaete males are mated to duplex ski females, the following distribution is obtained:

1 star dichaete : 1 star ski : 1 dichaete : 1 ski.....(4)

This ratio conforms to ratio (2) above, and might be taken to indicate that ski is a recessive character determined by a gene located in the third group.

Section 1 of table 3 contains the results of five cultures which conform to this distribution. The star dichaete males of the four D cultures were secured from a mating of duplex ski ♀ × star black sepia dichaete ♂, while that of culture 92 was from star dichaete ♀ × duplex ski ♂. The duplex ski individuals were taken from a proven stock established in the manner outlined below.

We have, however, pointed out above that it is possible to isolate a race of wild-type flies recessive to ski. The fact that this race is recessive to ski would naturally be interpreted as due to the fact that it bears the recessive third-group gene upon which the distribution obtained in ratio (4) depends. The conclusive evidence that a second-chromosome dominant is necessary for the production of ski is afforded by back-crosses of F_1 star dichaete males of the same constitution as those used in the above experiment to females of the wild-type recessive to ski. The results secured conform to the following distribution:

1 star dichaete : 1 star : 1 dichaete : 1 ski.....(5)

It will be observed, therefore, that a single second chromosome derived from the duplex ski race is sufficient to produce the ski character when the third-chromosome gene is homozygous. When it is heterozygous, however, as is shown by the dichaete classes of both ratios (4) and (5), the character is not produced whether the individuals be heterozygous or homozygous for the second-chromosome gene.

TABLE 3

Results of matings devised to prove the bigenic nature of ski.

SECTION NUMBER AND DESCRIPTION		*SD*	*SSi**	*S*	*D*	*Si*	+	TOTAL
1. P_1 *SD*× duplex S_i Duplex S_i♀♀ × F_1 *SD*♂♂ (Back-cross)	D2	59	48	.....	34	36	.....	177
	D3	63	41	.....	52	51	.....	207
	D4	64	41	.....	53	43	.....	201
	D5	54	38	.....	27	36	.....	155
	92	45	43	.....	26	45	.....	159
	Total	285	211	.....	192	211	.....	899
2. P_1 *SD*× duplex S_i s_i - + ♀♀ × F_1 *SD*♂♂ (Back-cross)	93	84	.....	99	88	67	.....	338
	94	59	.....	48	79	55	.....	241
	95	112	.....	100	75	84	.....	371
	97	90	.....	70	101	84	.....	345
	Total	345	.....	317	343	290	.....	1295
3. P_1 *SD*× duplex S_i Simplex S_i♀♀ × F_1 *SD*♂♂ (Back-cross)	124	48	25	28	50	51	.....	202
	125	57	39	33	84	69	.....	282
	Total	105	64	61	134	120	.....	484
4. P_1 *SD*× s_i - + Duplex S_i♀ × F_1 *SD*♂ (Back-cross)	121	37	56	.....	44	35	.....	172
5. P_1 *SD*× s_i - + Simplex S_i♀♀ × F_1 *SD*♂♂ (Back-cross)	122	102	36	30	87	42	46	343
	123	95	60	43	94	27	54	373
	132	113	46	54	132	43	58	446
	Total	310	142	127	313	112	158	1162
6. P_1 *SD*× s_i - + s_i - + ♀♀ × F_1 *SD*♂♂ (Back-cross)	113	77	.....	96	89	.....	87	349
	131	87	.....	77	114	.....	96	374
	Total	164	.....	173	203	.....	183	723

* The symbol S_i is also used to denote the *character* ski as it occurs in tables and matings, and the symbol s_i - + is used to denote s_i-wild-type in matings.

Section 2 of table 3 contains results of back-crosses of F_1 star dichaete males of the same constitution as those used in section 1 to s_i-wild-type females. The star dichaete males used in these cultures were derived from the same source as the one used in culture 92, and the s_i-wild-type females were taken from a stock culture.

The fact that ski is not a monogenic character dependent upon a single recessive third-chromosome gene, as might be concluded from ratio (4),

may be demonstrated directly by mating *inter se* the star ski segregants from appropriate cultures. The results from three pairs of such matings of star ski segregants from culture 92 are recorded in table 4. If the charac-

TABLE 4

Star ski segregants from culture 92 mated inter se.

	S	SS_i	S_i	TOTAL
128	9	132	66	207
144	13	111	69	193
156	15	183	90	288
Observed	37	426	225	688
Expected	*61*	*398*	*229*	*688*

ter were simply determined by a third-chromosome gene, the progeny should all be ski in the ratio of 2 star ski : 1 ski. The actual results, however, demonstrate the presence of a class of star flies. The interpretation of the proportion of star flies actually obtained must, of course, take into account absence of crossing over in the males, the lethal effect of star when homozygous, and the percentage of recombinations of S and S_i in the females. Assuming for this last item a value of 26.6 percent, which is that obtained in experiments for the determination of the locus of S_i, expectation calls for the following ratio:

86.7 star ski : 50.0 ski : 13.3 star.....(6)

This ratio is in fair agreement with the actual results secured, although there is a deficit in the star class.

From these results we conclude that ski differs from wild-type in two pairs of genes. The third-chromosome gene is recessive to its normal allelomorph and the one in the second-chromosome is dominant. There is no recessive lethal effect associated with the dominant gene, as is true of most of the dominant mutant genes in Drosophila. The consistent results secured further demonstrate that the ski genotypes are invariably ski in appearance, consequently we have no difficulties of somatic variation to deal with in working with it.

ESTABLISHMENT OF DUPLEX SKI STOCKS UNDER CONTROL

The accurate analysis of ski and the location of its genes depends upon the establishment of ski stocks known absolutely to be duplex. In our original material simplex and duplex ski flies were usually mixed together,

so that a great variety of results was secured from experiments with them.

The constitution of a ski fly may be determined by crossing it to members of a wild-type stock which has descended from ski parents. If the ski fly is simplex the progeny of such a mating will consist of equal numbers of ski and wild-type flies, whilst if it is duplex, the population will consist entirely of ski flies. It is not usually feasible, however, to test females before making the desired matings with them, consequently matings have been devised in which the progeny fall into diagnostic classes; i.e., classes the individuals of each of which belong to a known genotype. By double-mating males which prove to be duplex ski with star dichaete females it is possible to be assured of the production of F_1 star dichaete males of the constitution, $\frac{S}{S_i}\ \frac{D}{s_i}$. Such star dichaete males mated to duplex ski females give ratio (4) above, a practical demonstration of which is afforded by the results contained in section 1 of table 3. If, however, a selected female should happen to be simplex ski, the distribution among the progeny would conform to a different ratio, namely,

2 star dichaete : 1 star : 1 star ski : 2 dichaete : 2 ski.....(7)

The splitting of the star class into two sub-classes, star and star ski, definitely sets this ratio apart from the previous one.

An actual demonstration of conformance to ratio (7) is given in section 3 of table 3. The star dichaete males in this particular instance were not secured in the direct manner employed in the previous cases, but were taken from cultures in which the star dichaete males were known to be of the desired constitution. In culture 125 the star dichaete male was taken from culture 92, and the one used in culture 124 was derived from an uncounted culture with a history identical with that of culture 92. The simplex ski female of culture 124 was taken from culture 95, that of culture 125 from culture 93, in both of which cultures, as may readily be determined, all the ski segregants must have been simplex in constitution.

At the risk of being tedious the analysis must be carried further in order to point out some relations not shown in the above illustrations. For example, the necessity for determining the constitution of the original ski males and for selecting only those which prove to be duplex, may be demonstrated by considering the consequence of employing simplex ski males in the above manner. When a star dichaete female is mated to a simplex ski male the F_1 star dichaete segregants are of two genotypes, namely, $\frac{S}{S_i}\ \frac{D}{s_i}$ and $\frac{S}{\ }\ \frac{D}{s_i}$. The results of mating males

of the former genotype to duplex ski females have been discussed and illustrated above, but males of the latter mated to duplex ski females will give progenies conforming to precisely the same distribution, except that the ski segregants are all simplex! Ratio (4), therefore, is not assurance of the production of a duplex ski class unless the star dichaete parent is known to have been $\frac{S}{S_i}\ \frac{D}{s_i}$. As an experimental demonstration of this case, we have only the single culture recorded in section 4 of table 3. The star dichaete male was secured from s_i-wild-type ♀ × star dichaete ♂, both parents from stocks, and the duplex ski female used in the back-cross was also from a stock culture.

Results from matings of this latter type of star dichaete male with simplex ski females and with s_i-wild-type females are contained in sections 5 and 6, respectively, of table 3. The star dichaete males in section 5 were obtained from the same culture which produced the one used in culture 121, and the simplex ski females were again taken from cultures 93 and 95. The results here are clearly in agreement with the expected ratio:

2 star dichaete : 1 star : 1 star ski : 2 dichaete : 1 wild-type : 1 ski. . . (8)

The star dichaete males used in section 6 were also derived from the same culture as those used in sections 4 and 5, and in both cases they were mated to s_i-wild-type females from stock. No ski flies were produced. The results of this mating have been included merely for the sake of completeness. In agreement with expectation, the results conform to the general F_1 star dichaete ratio:

1 star dichaete : 1 star : 1 dichaete : 1 wild-type. . . . . (9)

The results should, however, be compared with those contained in section 2. In the controlled production of duplex ski, it would obviously be feasible, if desired, to test a mixed lot of F_1 star dichaete males by mating them to s_i-wild-type females rather than to test the original males, but a double mating can not be avoided at some stage in the procedure.

In a few cases we have made use of the method of testing both males and females by mating them to s_i-wild-type flies. In testing a ski female in this fashion she is first mated to an s_i-wild-type male. The male is left with her until she begins to produce eggs after which he is removed, and the female sub-cultured without the male until exhausted of all fertile eggs. She may then be mated to a duplex ski male which has previously himself been tested with an s_i-wild-type female. Those cultures, the female

parents of which gave only ski progeny in the test with s_i-wild-type, may then be retained for the establishment of duplex ski stocks. While this method is more direct than the one first outlined, it is rather uncertain in operation on account of the difficulty of keeping the females alive long enough to make the two matings. The work of NONIDEZ (1920) may be taken to indicate that the method is satisfactory provided that sufficient care is exercised to exhaust the tested females of all fertilized eggs before they are again mated. The objection that one can not always be sure that the female no longer contains a few spermatozoa from the previous mating may be met by reversing the procedure, i.e., mating females to known duplex ski males first and then testing them against s_i-wild-type males. In any case where this method of establishing the duplex ski stocks has been used specific mention of it is made in the text.

There are a number of other ways, some of which will be indicated in what follows, of securing duplex ski flies under control; but those described have been used in most of the work. The work, of course, was greatly facilitated after the morphological differences between simplex and duplex ski flies had been discovered. When extremely recurved ski individuals with pronounced "crimps" in the wings are used for parents throughout, it is practically certain that they will prove duplex in constitution. It is, therefore, necessary to establish fewer cultures when such selection is practiced. However, it is not safe to rely entirely upon morphological expression as an index of germinal constitution, consequently all duplex ski stocks which have been used in critical experiments have been established under rigid control.

MATINGS GIVING VARIOUS RATIOS OF WILD-TYPE TO SKI

In view of the adequacy of the experiments designed to determine the genetic basis of ski, and the location of its genes, it is not necessary to demonstrate separately all the relations which might be shown by the various types of matings involving the genes S_i and s_i and their normal allelomorphs. We have been content to perform only a few such experiments as opportunity arose in the prosecution of what we considered the more important experiments.

Simplex ski flies mated *inter se* should produce 3 ski : 1 wild-type. This is the only mating of ski × ski which gives any wild-type segregants. The ideal method of demonstrating this ratio is to mate duplex ski with s_i-wild-type flies to produce simplex ski, and then to mate these F_1 simplex ski flies *inter se*. We have been content, however, in table 5 to present results from ski × ski matings of parents selected from our original stock

while it was still throwing wild-type segregants. Several of these matings gave nothing but ski offspring, hence they must have been either of the type simplex ski × duplex ski or duplex ski × duplex ski. All cultures which gave any wild-type segregants are included in table 5. Wild-type flies are, as usual, in excess of the expected proportion of 25 percent. In these particular cultures the condition may have been somewhat aggravated by the fact that only two five-day sub-cultures were established under each culture number, but later results secured by the two-day sub-culture method show no particular increase in the proportion of ski flies realized, to that expected. If we set the survival of wild-type at 100 percent, then the ski class in these cultures is, on the average, 80.8 percent of expectation.

TABLE 5

Results from simplex ski mated inter se.

	S_i	+	TOTAL
Sk3	95	49	144
Sk5	34	13	47
Sk10	122	45	167
Sk11	76	14	90
Sk29	136	70	206
Total	463	191	654

An exact reversal of the foregoing ratio, namely, 3 wild-type : 1 ski, is accomplished by mating wild-type individuals of the constitution, $\frac{S_i}{S_i}\ \frac{s_i}{s_i}$, *inter se*. The *modus operandi* in this case was as follows. From duplex ski ♀ × star black sepia dichaete ♂ an F_1 star male was taken and backcrossed to a duplex ski female. The progeny of this mating consisted of 55 star, 52 star ski, 32 ski, and 49 wild-type flies. Here the wild-type flies must necessarily be $\frac{S_i}{S_i}\ \frac{s_e}{s_i}$. The results of matings of four pairs of wild-type flies are given in table 6. The influence of better conditions of experimentation is strikingly reflected in the total yields and ratios obtained from these four cultures. In this series each culture number was represented by five two-day sub-cultures. Although the ski segregants were all duplex in this case the attained values reach a percentage of 86.6 when compared with non-ski as 100 percent. In fact the number of ski individuals is slightly in excess of that of sepia, although these two classes should, theoretically, be present in equal numbers.

From the above cultures four test matings were made of ski × ski. All produced only ski descendants. This set of experiments was also partly instituted in order to secure a race of flies homozygous for S_i, but with the normal allelomorph of s_i. Since there is no crossing over in the male and not in excess of 15 percent of recombination of s_e and s_i in the female, it is evident that at least 85 percent of the sepia segregants in the above cultures are of the desired constitution. We started the desired stock, therefore, by mating sepia segregants *inter se*. This stock, which we call the S_i-sepia stock, has been carried on for over a year in mass cultures without producing a single ski segregant. Consequently its constitution may safely be considered as that desired.

TABLE 6

Wild-type $\left(\frac{S_i}{S_i}\ \frac{s_e}{s_i}\right)$ *mated inter se.*

	+	s_e	S_i	TOTAL
C7	260	113	111	484
C8	282	126	111	519
C9	241	76	119	436
C10	269	92	80	441
Total	1052	407	421	1880

The possession of S_i-sepia and s_i-wild-type stocks placed us in a position to reconstruct ski from two stocks breeding true for the normal wing type. Accordingly we made up a culture of S_i-sepia ♀ × s_i-wild type ♂, and one of the reciprocal. The two cultures yielded 535 wild-type flies. Two F_2 cultures were started with F_1 flies from the former of these two cultures. The results are recorded in table 7. It is clear from these results that ski flies may be obtained by crossing two stocks indistinguishable in appearance from wild-type. The expected ratio, 9 wild-type : 4 sepia : 3 ski, is not, however, well realized. As a matter of fact, ski flies occur in a proportion of only 35.8 percent of expectation, assuming as before 100 percent as the value of the non-ski classes. It is probable that this result is due to the unusually high production of these cultures, which favored elimination of the later-emerging ski flies by over-crowding in the culture bottles. The discrepancy in the ratio is practically confined to the ski class, for sepia flies occur in 97.1 percent of expectation, setting the wild-type class at 100.

We have also had occasion to make crosses of F_1 wild-type flies from duplex ski × wild-type, therefore, $\frac{S_i}{-}\ \frac{s_i}{-}$, in constitution, back to duplex ski flies. In this case the back-cross should give a ratio of 1 wild-type : 1 ski. The results from three cultures are given in table 8. They show a survival value of ski of 84.7 percent compared with the wild-type class.

TABLE 7

P_1, S_i-sepia ♀ × s_i-wild-type ♂. F_1 wild-type mated inter se.

	+	*si*	*Si*	TOTAL
153	385	157	55	597
154	333	153	30	516
Total	718	310	85	1113

The cultures are also interesting because they show such a marked decrease in total production as compared with the other series of cultures reported in this section. A lowered productivity appears to be associated constantly with our duplex ski races, and to a somewhat lesser extent with simplex ski, so that some extra attention must often be given the stocks in order to keep them in a thriving condition.

TABLE 8

P_1, duplex ski ♀ × wild-type ♂. F_1 duplex ski ♀ × wild-type ♂.

	+	*Si*	TOTAL
B1	76	72	148
B3	92	86	178
B4	101	70	171
Total	269	228	497

A considerable number of other matings could be readily devised to illustrate interesting relations of ski contrasted with various wild-type genotypes. It is not, however, necessary to go through the complete set of them in order to demonstrate adequately the mode of inheritance of ski. What is important, however, in this connection, is a realization of the fact that every mating may be made under perfect control, so refined are the modes of analysis in Drosophila. It is not necessary in Drosophila, as is often the case in other forms, to conjecture the genotypes involved in a particular mating from the results of that mating. It is perfectly possible

and feasible to know before the matings are made what the genotypes of the parents are, and what results should be secured from them.

DETERMINATION OF THE LOCUS OF SKI-II

The determination of the loci involved in a bigenic character presents no particular difficulty, save that the establishment of appropriate stocks is a tedious and troublesome task. At the outset we decided to employ the three-point method of locating each gene and to carry out the four necessary complementary back-crosses in order to balance the inviability of ski, which we at first thought was rather marked.

For the determination of the locus of S_i, star and black were used as reference points. They were the only suitable second-chromosome genes which we had represented in our stocks; and, fortunately for the accuracy of the determinations, S_i was found to lie between them. As far as we know, no two better reference points could have been chosen, because there is absolutely no interference in the expression of the three characters in any combination. In experiments for the location of S_i it is necessary to have all stocks homozygous for s_i so that ski will appear whenever S_i is present. Since S and S_i are both dominant genes, the back-cross takes in each case the form of F_1 star simplex ski ♀ × black-s_i ♂. In order to perform the four complementary back-crosses, the eight types of flies listed below were necessary:

Star-s_i	$= \frac{S}{\quad} \; \frac{s_i}{s_i}$
Star ski	$= \frac{S \; S_i}{S_i} \; \frac{s_i}{s_i}$
Star black-s_i	$= \frac{S \quad b}{b} \; \frac{s_i}{s_i}$
Star ski black	$= \frac{S \; S_i \; b}{S_i \; b} \; \frac{s_i}{s_i}$
Ski	$= \frac{S_i}{S_i} \; \frac{s_i}{s_i}$
Ski black	$= \frac{S_i \; b}{S_i \; b} \; \frac{s_i}{s_i}$
Black-s_i	$= \frac{b}{b} \; \frac{s_i}{s_i}$
s_i-wild-type	$= \frac{\quad}{\quad} \; \frac{s_i}{s_i}$

It is not necessary to establish stocks of all these different types, for obviously each of the four different star stocks will give the corresponding one of the non-star types by segregation. It was found more satisfactory,

however, in some cases to have actual stocks of the non-star types on hand rather than to depend upon segregation in star stocks for them. Pertinent details in the establishment of the stocks will be included in the discussion of the individual back-cross experiments.

Results from the $\frac{S}{S_i\ b}$ back-cross are recorded in table 9. The F_1 star ski flies were produced from the mating, star-s_i ♀ × ski black ♂. The star-s_i stock had been secured from an original mating of star dichaete ♀ × duplex ski ♂. From such a mating the F_1 star dichaete flies, mated *inter se*, produce, among other classes, a star ski class, the members of which are mostly simplex for ski. Such F_2 star ski flies, mated *inter se*, produced the desired star-s_i flies as an F_3 segregation product. The ski black stock was established by mating together the F_2 ski black individuals

TABLE 9

P_1, star-s_i ♀ × ski black ♂. Back-cross, F_1 star ski ♀ × black-s_i ♂.

	S	*Sib*	*SSib*	+	*Sb*	*Si*	*SSi*	*b*
189A	122	94	41	53	14	31	18	6
189B	107	82	27	36	21	20	5	2
189C	99	60	39	41	15	26	16	1
189D	56	55	12	28	3	14	5	1
Total*	384	291	119	158	53	91	44	10
	107	82	27	36	21	20	5	2

* The totals carried forward to table 13 are stated on the second line. They represent the results of culture 189B only.

from an original cross of duplex ski ♀ × black ♂. In this case the F_2 ski black segregants are all simplex and the F_3 progenies conform to the ratio, 1 duplex ski black : 2 simplex ski black : 1 black-s_i. From among the ski black segregants individuals of both sexes were selected which had the most strongly recurved and conspicuously "crimped" wings, and tested by mating them to black-s_i flies. In each case the progenies consisted entirely of ski black flies. The tested ski black females were then exhausted of all fertile eggs and subsequently mated to tested ski black males for the establishment of the duplex ski black stock. While there is an element of uncertainty, as we have stated before, in the testing of females, no difficulty can arise from it here, because the production of F_1 (star and non-star) ski progeny from star-s_i ♀ × ski black ♂ is absolute assurance that the F_1 star ski females are of the proper constitution for use in the

back-cross test. A black-s_i stock was established at the same time from the F_3 black-s_i flies.

An examination of the data from these four cultures shows that three of them suffer from marked disproportions especially in the star black and ski and in the star ski and black classes. Culture 189B is free from these discrepancies. Since like irregularities do not appear in subsequent data, except in one culture, it would seem that these three cultures have not yielded normal results for the back-cross, but indicate the existence of some undetermined disturbing agent. They are, therefore, not included in the total carried forward to table 13, even though most of the results of their irregularity would be swamped in the data of that table.

The results of the $\frac{S \quad S_i}{b}$ back-cross are recorded in table 10. The F_1 star ski flies were produced from the mating, star ski ♀ × black-s_i ♂. The star ski stock was established from the same line of cultures which was the source of the star-s_i stock. It was not established as a duplex ski stock, but was merely maintained by selecting star ski flies for the production of each successive generation. In order to produce the F_1 females for the series of cultures included in table 10, selected star ski males were mated to black-s_i females. F_1 star ski females were then taken from a culture which produced nothing but star ski and ski flies.

TABLE 10

P_1, star ski ♀ × black-s_i ♂. Back-cross, F_1 star ski ♀ × black-s_i ♂.

	SS_i	b	Sb	S_i	SS_ib	+	S	S_ib
190A	141	100	50	55	25	23	3	3
190B	161	166	63	71	30	34	8	6
Total	302	266	113	126	55	57	11	9

The results of the $\frac{S \quad S_i \quad b}{\quad}$ back-cross are contained in table 11. The F_1 star ski females were produced from the mating, star ski black ♀ × s_i-wild-type ♂. For the establishment of star ski black stock, star ski black males were taken from culture 190A. They must obviously have been of the constitution $\frac{S \quad S_i \quad b}{b} \; \frac{s_i}{s_i}$. They were mated to stock ski black females, whereby the progeny was divided equally between two classes, star duplex ski black and simplex ski black. The former, mated *inter se*, were the foundation for the star ski black stock. The s_i-wild-type stock

had been established in the usual manner by taking the wild-type segregants from simplex ski flies mated *inter se.*

The results of the $\frac{S \quad b}{S_i}$ back-cross are recorded in table 12. The F_1 star ski females were produced from the mating, star black-s_i ♀ × duplex ski ♂. The establishment of the star black-s_i stock was effected by simply mating star black segregants from culture 190A *inter se.* The duplex ski stock was the original stock from which all the stocks mentioned in this account were derived.

TABLE 11

P_1, star ski black ♀ × s_i-wild-type ♂. Back-cross, F_1 star ski ♀ × black-s_i ♂.

	SSib	+	*S*	*Sib*	*SSi*	*b*	*Sb*	*Si*
195A	145	176	60	64	35	32	2	3
195B	97	112	37	31	12	12	1	3
195C	71	69	37	30	15	18	..	3
195D	73	79	30	32	12	7	..	2
195E	99	104	30	39	18	22	2	..
195H	48	76	34	29	14	6	..	1
195K	71	73	32	26	13	16	2	..
195L	97	108	51	38	25	15	2	3
Total	701	797	311	289	144	128	9	15

TABLE 12

P_1, star black-s_i ♀ × ski ♂. Back-cross, star ski ♀ × black-s_i ♂.

	Sb	*Si*	*SSi*	*b*	*S*	*Sib*	*SSib*	+
196A	71	83	28	36	12	14	2	1
196B	72	57	35	26	21	14	11	4
196C	90	76	38	41	17	27	1	1
196D	13	61	21	61	64	4	1	31
196G	50	34	16	18	2	5	1	..
196H	89	95	43	30	23	23	1	4
Total*	385	406	181	212	139	87	17	41
	372	345	160	151	75	83	16	10

* The totals carried forward to table 13 are stated on the second line. They do not include the results of culture 196D.

In culture 196D there are marked disproportions in all the four pairs of classes, which indicates that the results belong to a different class of data from those given by the other five cultures of this series. Here again

the nature of the disturbance was not determined, but the results from this culture are not included in the totals carried forward to table 13.

We have been content to outline the mode of procedure which was actually employed in the experiments, leaving to the reader's ingenuity the problem of determining for himself how it might have been modified to avoid establishment of so many complex stocks. Such modification is perfectly feasible, if advantage be taken of the exact knowledge of the genotype of every individual in a given back-cross population.

A summary of the results secured from the four complementary back-crosses, excluding the aberrant cultures which have been noted in the discussions of the corresponding tables, is contained in table 13. Similar tables containing the percentages of recombinations for each of the

TABLE 13

Summary of the results of the four complementary back-cross experiments giving data on the relations of S, S_i and b.

COMBINATIONS	—	-|-	—|-	-|-|-	TOTAL	RECOMBINATION PERCENTS		
						S, *Si*	*Si*, *b*	*S*, *b*
$\frac{S}{S_i \quad b}$	189	63	41	7	300	23.3	16.0	34.7
$\frac{S \; S_i}{b}$	568	239	112	20	939	27.6	14.1	37.4
$\frac{S \; S_i \quad b}{}$	1498	600	272	24	2394	26.1	12.4	36.4
$\frac{S \quad b}{S_i}$	717	311	158	26	1212	27.8	15.2	38.7
Total	2972	1213	583	77	4845	26.6	13.6	37.1

several cultures were prepared but they are not included in this account. Aside from the aberrant cultures, the amount of variation shown in the values calculated from the separate cultures is no greater than that met with normally. For the 4845 flies included in table 13 direct computation gives recombination values of 26.6 percent for *S* and S_i, 13.6 for S_i and *b*, and 37.1 for *S* and *b*. The direct value given by BRIDGES and MORGAN (1919) for *S* and *b* is 37.9, based on 16,507 flies. The agreement, therefore, is satisfactory. The coincidence of crossing over, based upon the totals of table 13, is 43.8 percent, calculated as follows:

$$\frac{77 \times 4845 \times 100}{1290 \times 660} = 43.8 \text{ percent}$$

In determining the probable locus of S_i from these data it is still necessary to allow for a certain amount of undetected double crossing over in the two sections of the chromosome under consideration. Approximate corrections can be made by proper substitution in the formula proposed by BRIDGES and MORGAN (1919), namely,

$$x^2 - \frac{x}{c} = -\frac{o}{2c}$$

In this equation, x is one-half the corrected distance, c is the coincidence, and o is the observed recombination value, all stated as decimal fractions. For the probable value of the coincidences in these two sections we have taken values assumed by BRIDGES and MORGAN for similar sections of the chromosome. Thus they correct their dachs-black data from a direct value of 17.8 percent to 18.5 on the basis of an assumed value of 50 percent for coincidence. This region is comparable to our S_i-b region, the direct value of which, 13.6 percent, must then be corrected to 14.1, assuming the same value for coincidence. Similarly they assign a value of 30 percent for the coincidence in the star-dachs region, the direct value of which, 27.3 percent, is comparable to our S-S_i value. S-S_i, then, with a direct value of 26.6 percent, is corrected to 27.8 on the basis of an assumed coincidence of 30 percent. In view of the low coincidence value actually obtained from our data, objections may be raised to the high values which have been used in correcting them; but, since the values are proportional, this fact probably does not result in any appreciable error in the calculations. We then have $27.8+14.1=41.9$ as the value for the star-black distance. The mean value which BRIDGES and MORGAN employ for this distance, based on all pertinent data for the second-chromosome genes, is 46.5, a value which exceeds our determination by 4.6 percent. This discrepancy must be taken into account in the final assignment of a proper locus for S_i in terms of the map which they have constructed for the second-chromosome genes. If the difference is divided in proportion to the lengths of the two sections involved, the final value for S_i becomes 30.8, a position slightly to the right of dachs. S_i does not, therefore, occupy any hitherto unrepresented region of the second chromosome. The ease of classification of the character and its dominance make the mutant one to be considered in genetical studies in which this region is followed. The fact that all the flies used in the final matings of such experiments must be homozygous for s_i adds a few generations or a few cultures to the preparatory work, which is not a serious handicap in Drosophila.

DETERMINATION OF THE LOCUS OF SKI-III

Preliminary investigations indicated that the loci for dichaete and spineless would provide suitable reference points for the determination of that of s_i. Accordingly stock cultures of duplex ski spineless and s_i-spineless were made up in the customary manner. The establishment of an s_i-spineless race presents no difficulties, because any spineless segregants from ski parents will necessarily be of this type. In the establishment of duplex ski spineless, however, it is necessary, after ski spineless flies are obtained, to follow the method previously outlined in this paper in order to assure ourselves of the duplex character of the stock. The duplex ski spineless stock has been maintained for nearly two years in mass cultures without producing a single wild-type fly.

The details pertinent to the performance of each of the four types of mating are discussed below. It seems desirable, however, to describe at this point the general method which was used in the prosecution of the experiments. In the preceding set of experiments for the determination of the locus of S_i, ten cultures were started for each type of mating, a total of forty cultures for the entire series of experiments, but only twenty of them were successful. The failures resulted apparently mostly from unfavorable bacterial growths, which are often transferred from bottle to bottle with the parents, if they once become established, and to the necessity for using ski females in the back-crosses. In the S_i series of cultures no attempt was made to replace unsuccessful cultures. This accounts for the discrepancy in the numbers of cultures secured in the different back-crosses. In the s_i series of cultures, it was decided at the outset to replace all unsuccessful cultures until results from ten cultures were secured from each back-cross. In order to do this with the minimum amount of labor, stock cultures were established from which virgin females of proper constitution for use in back-crosses could be isolated at any time. Since in every case the back-cross takes the form, dichaete ♀ × duplex ski spineless ♂, these stocks were maintained by successive matings of dichaete males with appropriate females. The following list enumerates the various matings which were necessary for maintaining these stocks:

(1) $\frac{\quad}{\quad}$ ♀ × $\frac{D \quad s_i \quad s_s}{\quad}$ ♂

(2) $\frac{\quad \quad s_s}{s_s}$ ♀ × $\frac{D \quad s_i}{s_s}$ ♂

(3) $\frac{s_i}{s_i}$ ♀ × $\frac{D \quad s_s}{s_i}$ ♂

(4) $\frac{s_i \quad s_s}{s_i \quad s_s}$ ♀ × $\frac{D}{s_i \quad s_s}$ ♂

The last stock in this list was never established in this manner, but it is included for the sake of completeness. The actual experiments with this combination were carried out in a different manner, which is fully described below. It is understood that S_i is present in none of these stocks. Each of the stocks obviously segregated in each generation for the two classes of flies represented by the parents, and once established they could easily be maintained by mating virgin non-dichaete females to dichaete males. When it was desired to make a back-cross test, it was merely necessary to take out virgin dichaete females from the appropriate stock and mate them to duplex ski spineless males. The manner in which the original stocks were established is described in connection with the account of the experiments in which they were used.

The series of cultures for the $\frac{D}{s_i \quad s_s}$ combination was carried out first. It differs in method from the other series of cultures in that the gene, S_i, was here introduced from the female side, so that the actual matings for the linkage determinations were of the type, $\frac{S_i}{S_i} \quad \frac{D}{s_i \quad s_s}$ ♀ × — $\frac{s_i \quad s_s}{s_i \quad s_s}$ ♂. This type of mating is of some additional interest because both parents are non-ski in appearance, yet on crossing them the progeny is distributed in a ratio of 1 non-ski : 1 ski.

In order to secure dichaete females of the above type, a duplex ski spineless female was mated to a star dichaete male. An F_1 star dichaete male from this mating was then back-crossed to a duplex ski spineless female, whereby a progeny conforming to the following ratio was obtained:

1 star dichaete : 1 star ski spineless : 1 dichaete : 1 duplex ski spineless.

Here all the non-star flies must be homozygous for S_i. Dichaete females were, therefore, isolated and mated to s_i-spineless males with the results contained in table 14. This method is needlessly complex, for, given an s_i-spineless stock, the obvious mode of operation would be to mate individuals of it to dichaete and then to back-cross the F_1 dichaete females to duplex ski spineless males. The method adopted was followed merely in order to show that the determinations could be made satisfactorily in this manner.

The series of cultures for the $\frac{D \quad s_i \quad s_s}{}$ combination, included in table 15, has the following history. A dichaete ski spineless male from culture

H11 (cf. table 14) was taken as the starting point. He must obviously have been of the constitution, $\frac{S_i}{\quad}\ \frac{D\ s_i\ \ s_s}{s_i\ \ s_s}$. It was necessary for the establishment of the proper stock, (1) in the list given above, to get rid of the second chromosome bearing S_i, and the third containing s_i and s_s. In order to do this the selected dichaete ski spineless male was out-crossed to a star female. The progeny secured from this mating consisted of the

TABLE 14

Results of back-crossing dichaete $\left(\frac{S_i}{S_i}\ \frac{D}{s_i\ \ s_s}\right)$ *females to* s_i*-spineless males.*

	D	Sis_s	$DSis_s$	+	Ds_s	Si	DSi	s_s
H11	193	199	9	12	22	21	3	2
H12	159	197	12	4	31	23	1	2
H13	144	128	3	9	22	11	..	3
H15	63	62	6	2	11	15	..	1
H16	51	36	2	7	10	5	1	..
H17	152	137	5	10	17	13	..	..
H18	138	161	10	4	19	14	2	2
H19	176	175	8	14	28	23	2	3
H20	50	55	3	5	11	9	..	4
H21	112	113	5	7	16	18	1	4
Total	1238	1263	63	74	187	152	10	21

usual four classes (ratio (1)), but the star dichaete flies might be either $\frac{S}{S_i}\ \frac{D\ s_i\ \ s_s}{\quad}$ or $\frac{S}{\quad}\ \frac{D\ s_i\ \ s_s}{\quad}$. A star dichaete male from this culture was then mated to a black sepia female (the black and sepia characters have no significance here) and following this mating star dichaete males were out-crossed for four consecutive generations to stock wild-type females until it was desired to use the stock thus established for linkage determinations. Star was finally eliminated by simply selecting dichaete males for the continuation of the stock. The dichaete flies of these latter generations could have been only $\frac{D\ s_i\ \ s_s}{\quad}$ in composition. The cultures of table 15 were established from such dichaete females mated to duplex ski spineless males.

While it is not absolutely necessary to have females free from S_i in these experiments, it is nevertheless desirable not to introduce this gene from

the female side in order to avoid the production of duplex ski segregants, which are probably of lower viability than simplex ski flies. In matings carried out in the manner described, all segregants will obviously be heterozygous for S_i, thus avoiding any difficulties which might arise, if there were differences in this respect.

The series of cultures for the $\frac{D \quad s_i}{s_s}$ combination was followed out in the same general manner as that for the one just described. The starting point was a dichaete ski male, obviously $\frac{S_i}{} \quad \frac{D \quad s_i}{s_s}$, from culture H21 (cf. table 14). He was out-crossed to a stock star female, and then star dichaete males were out-crossed in consecutive generations to wild-

TABLE 15

Results of back-crossing dichaete $\left(\frac{D \quad s_i \quad s_s}{}\right)$ *females to duplex ski spineless males.*

	$DSis_s$	+	D	Si_{Ds}	DSi	s_s	Ds_s	Si
N13	97	107	3	1	14	5	..	..
N14	140	173	4	4	14	17	1	..
N15	140	250	10	11	20	20	2	1
N16	57	128	3	1	1	12	1	1
N17	70	114	4	4	8	4	..	1
N18	161	202	6	5	20	16	3	..
N19	136	199	4	6	18	17	3	1
N20	135	275	11	8	14	25	1	..
N21	112	134	6	9	5	17	..	1
N22	133	173	6	3	21	12	1	..
Total	1181	1755	57	52	135	145	12	5

type females until the original second and third (non-dichaete) chromosomes were replaced. As a matter of fact these matings were carried on for seven generations, after which the stock was maintained ready for use at any time by mating dichaete males in successive generations to spineless females. Dichaete females were isolated from this stock from time to time and mated to duplex ski spineless males until the desired number of successful matings had been secured. The results are recorded in table 16.

The starting point of the $\frac{D \quad s_s}{s_i}$ combination was a dichaete spineless male, obviously $\frac{S_i}{} \quad \frac{D \quad s_s}{s_i \quad s_s}$, from culture H12 (cf. table 14). He

TABLE 16

Results of back-crossing dichaete $\left(\frac{D \quad s_i}{\quad s_s}\right)$ *females to duplex ski spineless males.*

	DSi	s_s	Ds_s	Si	$DSis_s$	+	D	Sis_s
6	220	260	5	12	17	24	1	1
7	242	323	12	12	18	31	..	2
8	246	356	9	9	25	32	..	1
9	143	165	13	10	14	30	2	3
10	150	180	6	3	11	22	1	..
13	261	289	12	7	28	26	1	3
42	153	157	4	7	17	21	1	1
50	267	330	17	8	38	26	4	1
51	118	124	3	3	16	10	..	..
74	67	117	..	6	7	10	2	1
Total	1867	2301	81	77	191	232	12	13

was mated to a star female from stock, and then star dichaete males were mated in successive generations to wild-type females until the stock was free from the original S_i and s_i-s_s chromosomes, after which the stock was maintained ready for use by mating dichaete males to s_i-wild-type females. Dichaete females from this stock were isolated for the establishment of the first eight cultures of table 17.

TABLE 17

Results of back-crossing dichaete $\left(\frac{D \quad s_s}{\quad si}\right)$ *females to duplex ski spineless males.*

	Ds_s	Si	DSi	s_s	D	Sis_s	$DSis_s$	+
35	155	155	2	6	15	17	..	..
39	161	162	6	9	26	9	..	..
40	89	101	1	4	10	13	1	1
56	152	164	18	19	24	21	4	2
57	132	136	10	13	21	21	2	2
58	134	172	16	16	25	13	1	1
59	79	70	..	2	5	8	..	..
60	140	195	9	17	23	24	..	2
177	304	266	11	17	29	31	2	3
179	223	209	11	15	31	21	..	2
180	328	246	8	24	35	39	3	3
Total	1897	1876	92	142	244	217	13	16

This stock was carelessly permitted to run out before the desired number of successful cultures had been secured from it. The females of the

last three cultures of table 17 were, therefore, derived from a different source. A spineless female was mated to star dichaete male, both from stock. An F_1 star dichaete female was then mated back to a spineless male, and from the progeny a dichaete spineless male was selected and mated to an s_i-wild-type female. Dichaete females produced from this mating were then mated to duplex ski spineless males for the establishment of the last three cultures.

A summary of the data obtained from these four sets of cultures is contained in table 18. In calculating the final values for the locus of s_i we have used the totals given at the foot of the table. This method is equivalent to an assignment of weights proportional to the number of individuals counted for each combination, but it appears preferable to all other possible ones because it calls for a minimum amount of manipulation of the raw data obtained from the experiments. It may be noted in passing, however, that other methods of computation do not materially alter the values recorded in table 18. Thus, if each set of cultures is given

TABLE 18

Summary of the results of the four complementary back-cross experiments giving data on the relations of D, s_i and s_3.

COMBINATIONS	—	+—	—+	++	TOTAL	RECOMBINATION PERCENTS		
						D, s_i	s_i, s_3	*D*, s_3
$\frac{D\ s_i\ s_3}{}$	2936	109	280	17	3342	3.8	8.9	11.6
$\frac{D}{s_i\ s_3}$	2501	137	339	31	3008	5.6	12.3	15.8
$\frac{D\ s_i}{s_3}$	4168	158	423	25	4774	3.8	9.4	12.2
$\frac{D\ s_3}{s_i}$	3773	234	461	29	4497	5.8	10.9	15.5
Total	13378	638	1503	102	15621	4.7	10.3	13.7

equal weight, the linkage values become 4.8, 10.4, and 13.8 instead of 4.7, 10.3, and 13.7, respectively, and if computations are based on non-ski classes only, following BRIDGES's (1921) suggestion for calculations involving a character of inferior viability, the values become 4.9, 10.4 and 13.9, which again differ only slightly from the values secured by direct computation.

In fitting these data to those which have already been presented, we have taken BRIDGES'S values, dichaete = 38.5 and spineless = 54.2, as representing the most probable values for our two reference points. The *D-s*$_s$ section should, therefore, be 15.7 units in length, instead of 15.0 as given by our data. It seems unnecessary to correct these short distances for coincidence, although we are working in the region of highest coincidence in the chromosome. Consequently a simple correction based on the division of the difference in proportion to the lengths of the sections involved gives a final value of 43.4 for the locus of s_i in terms of BRIDGES'S scale of values.

An examination of the data for s_i on the basis of coincidence discloses the existence of very high values throughout the series. The summarized data of table 17 give a coincidence value of 134.2 percent, when theoretically a very low value should be secured for such a short section of the chromosome. If the value is computed by taking the average of the values of the separate cultures, as suggested by WEINSTEIN (1918), a coincidence of 131.5 percent is secured, a value not differing significantly from the preceding one. We thought that errors in classification might have been responsible for the high values obtained, but an examination of the data from this standpoint has not led to the detection of any. We have, therefore, no definite explanation to offer for the high coincidence values secured in these cultures, but they seem to be real values not dependent upon errors of classification.

An interesting question is here raised as to the relation between s_i and c_u, the gene for curled. GOWEN (1919) places curled 3.7 units to the right of dichaete, but his mean value for the distance between dichaete and spineless is only 10.4, whereas later determinations raise this value to 15.7. If we correct his value for dichaete to curled proportionally, it becomes 5.6, which would place the locus of curled at 44.1, as compared with 43.4 for s_i. It might be possible, therefore, as far as these data are concerned, for s_i and c_u to be allelomorphic, although the fact that a wild-type F_1 is secured from duplex ski $\times$ curled argues strongly against this interpretation. We have also found that black flies of the constitution, $\frac{S_i \quad b}{S_i \quad b} \frac{s_i}{c_u}$, have wild-type wings; but we have not been able to secure final proof of non-allelomorphism through the establishment of a ski-curled race of the constitution, $\frac{S_i}{S_i} \frac{s_i \quad c_u}{s_i \quad c_u}$. Obviously, in view of the short distance between s_i and c this would be a difficult task, hardly worthy of the effort required. Some preliminary experiments carried out

with this object failed of success, doubtless because they were not extensive enough to yield the desired results.

VALUATION OF SKI

It has already been pointed out that ski flies are always readily separable from those with wild-type wings, and that flies genotypically ski are always of that phenotype. Ski is, therefore, available for use in any case where the contrast is between wild-type and ski wings, or for any mutant form involving other than wing characters. With some other wing characters such as curved and dichaete, it may also be used with a fair degree of accuracy, although an occasional misclassification is probably unavoidable. With more-marked wing characters, it probably cannot be used. For example, we have found that with miniature it produces simply a crumpling of the wing unsuitable for accurate classification.

The viability of ski depends intimately on cultural conditions. Typically members of the ski classes appear somewhat later than those of non-ski classes in the same cultures, so that care must be taken to retain cultures until all of the flies have emerged. Examination of individual sub-culture records shows that ski flies had hardly begun to emerge in many instances before the non-ski flies had practically all appeared. It would seem reasonable to suppose that in the event of unfavorable conditions in the medium, particularly such as restrict food supply, the later portions of the count would fail to appear, thus giving rise to discrepancies in the ski classes such as have been secured occasionally in the actual results. That these discrepancies are not indicative of corresponding gametic aberrancies is clearly indicated by the fact that often they did not occur, and the proper class relations have always been secured in analytical crosses.

A quantitative examination of the amount of disproportion in the ski classes shows that it is subject to wide fluctuations. Some figures have already been presented of the relation of ski observed to ski expected where the segregation was relatively simple; i.e., where there are only a few classes in the progeny. Here the percentage of survival ranges from 35.8 to 90.0 in different experiments. The data are not extensive enough or of the right kind to determine the relative survival value of simplex and duplex ski. They are, moreover, open to the objection that the wild-type flies are of different genotypes. While the evidence indicates no striking differences in viability between different wild-type genotypes; nevertheless exact relations have not been determined, and they would require a very extensive series of experiments.

A comparison of ski and non-ski frequencies in table 3, on the basis of non-ski observed = 100 percent of expectation, gives a survival value of 90.2 percent for ski, taking all the results together. A similar computation from the data of the S_i series of back-cross experiments gives a survival value of 93.6 percent, where equal numbers of non-ski and ski are expected. The s_i series of back-cross experiments gives a survival value of 85.4 percent. In this last series of cultures, a higher average yield per culture was secured. We have often observed that cultures giving a moderate number of flies conformed more closely to expectation than those in which the yield was unusually high. We may conclude that a survival value of 90 percent may be secured under reasonably favorable conditions.

As will be readily understood from the above accounts, the usefulness of ski for instructional purposes depends almost entirely upon the cultural methods adopted. If these are good, then ski furnishes unique material for demonstrations of factor relations of what might be termed the second order of difficulty, but if good cultural conditions cannot be provided, very marked discrepancies may occur in the ratios.

DISCUSSION OF RESULTS

Some questions of no little theoretical importance are raised by the origin of ski and its mode of inheritance. We have pointed out in our discussion of its origin that in all probability no wild strain entered into the cultures from which it was finally secured, but that it came from hybrid cultures derived from our laboratory wild-type and white-eyed stocks. It might, therefore, have represented merely a recombination of genes in these two stocks, assuming that one contained S_i and the other s_i, in the same manner that we have actually obtained ski from two stocks with wild-type wings. But individuals of these two stocks had been crossed frequently in order to demonstrate the mode of inheritance of the white-eye character, and no ski segregants had ever been observed. At the present time we do not have these two stocks in their original condition, for we have often reëstablished our white stock from out-crosses to other forms and we have unfortunately not been careful to retain our wild stock in an unbroken line, but have occasionally reëstablished it from wild sources. It is, therefore, impossible to determine by direct test what the original constitution of these two stocks may have been. Assuredly, however, the first appearance of ski in our cultures was due to segregation rather than to original mutation, as is shown by the number of ski individuals secured in the first cultures, by the fact that two parallel cultures produced the new form, and by the further *a priori* difficulty of assuming the simultaneous occurrence of two mutations in the same cultures with

such relations to each other. It seems certain that the mutations in both of the loci had occurred long previous to the actual appearance of the character, how long we can not even conjecture from our data.

It must be admitted that the manner of origin of ski and its mode of inheritance, considered superficially, lend some support to the oft-repeated contention that new characters arise by recombination rather than by actual mutation. While there are few geneticists who uphold this hypothesis in the extreme form in which LOTSY (1916) has developed it, nevertheless there are some who contend that undue stress has been laid upon the origin of new characters by mutation, and that many so-called mutations are nothing more than complex segregation phenomena. We have been led to give this matter particular attention through numerous discussions with the HAGEDOORNS,[3] who, while granting the occurrence of some "loss mutations," are disposed to credit the majority of cases of mutation to segregation. It is perfectly feasible to build up a plausible argument around this hypothesis, and it is doubtless true that the evidence in many cases may equally well be interpreted in either way. Thus in its simplest form, we may assume that two genotypes, in general *AAbb* and *aaBB*, exist, which are nearly, if not quite, identical in appearance. Upon hybridization an F_1 is produced which is like the parents in appearance, assuming that the *AB* phenotype is also identical with *Ab* and *aB;* but in the F_2, if the double recessive, *aabb*, represents a new form, one in sixteen of the individuals will be of a different type. In populations of ordinary size, the new form would appear in such small numbers that one would naturally be inclined to regard it as a mutant form, and subsequent tests with either parent form would strengthen this belief, for in both cases a simple monohybrid ratio would be secured. Should either of the parent forms have been discarded, in view of their phenotypic identity, it would be impossible to determine the true significance of the origin of such a "mutant." The criticism that often mutations have not been subjected to adequate test matings from this standpoint appears to us a valid one.

A similar line of argument is possible for ski. Assuming that we had at the beginning two strains of wild-type flies, one containing S_i and the other s_i, hybridization would result in the production of some ski individuals in F_2. They might appear in much below the normal ratio of 13 wild-type : 3 ski, as we have shown by direct tests of this mating. If, now, we had first established ski in a pure-breeding stock by continual selection,

[3] A full discussion of their position is contained in HAGEDOORN and HAGEDOORN Vorstheuvel LA BRAND (1921).

before seeking to determine its genetic significance, and in the meantime had discarded one of our original parent stocks, ski would behave as a simple recessive in case we had retained the S_i stock, or as a simple dominant in case the other stock had been retained. In either case the origin of ski might logically have been ascribed to mutation in a single locus of whichever parent stock had been retained, and the possibility of detecting the true significance of its origin would have been lost. The recombination hypothesis applies, therefore, as well and as simply to the origin of dominant characters as to that of recessive ones.

But while we have been led from our results with ski to point out these theoretical possibilities, we do not believe they constitute a valid explanation of the origin of the many mutations in Drosophila. In the first place we have direct evidence to the contrary from the study of ski itself. It is assumed in Drosophila that the wild type is not a conglomerate of different genotypes making up a single phenotype, but that its phenotypical uniformity, wherever found, is a manifest of a corresponding genotypic homogeneity. In the work with ski it has been necessary to use a large number of mutant stocks, presumably derived from a variety of sources, yet there seems to be no escape from the conclusion that all these stocks were uniform in constitution with respect to S_i and s_i, possessing neither of them but their normal allelomorphs. Had it been otherwise, it would be difficult to account for the consistent results which have been secured. It seems to us that the same line of argument applies to all the other simple mutant characters of Drosophila, whether dominant or recessive. They have been recombined in every conceivable fashion; they have been derived from a great variety of sources; and a great variety of different stocks has been kept on hand; yet they have always behaved in a manner consistent with the hypothesis that they are the results of actual point-mutation. Moreover, direct attempts to secure new forms by hybridization of wild strains derived from distinct sources have been uniformly disappointing. New forms are apparently as often produced by stocks which have been carried on for numerous generations in the laboratory without crossing as in those which are of hybrid origin. STURTEVANT (1921) has recently summarized and discussed the evidence on this point, not only for *melanogaster*, but also for several other species, consequently it is unnecessary to recite the details here. The only logical conclusion seems to be that, while the possibility of producing new forms by hybridization must be recognized, particularly in work with the polymorphic species of domesticated plants and animals, yet in Drosophila the genetic constitution of the various stocks is so well and completely known as to

preclude any general application of this hypothesis to it. It does not seem to matter, as far as the validity of these arguments is concerned, whether one subscribes to the theory of presence and absence or to the alternative one of actual change in the composition of the gene itself.

The mode of inheritance of ski has many analogies in other forms, so that any arguments which may be advanced for it may be applied by extension to such cases. In general any character relation such that a given form has two identical forms opposed to it one of which is dominant and the other recessive fulfills the conditions of parallelism. Thus CLAUSEN and GOODSPEED (1921) have recently demonstrated the existence in *Nicotiana Tabacum* of two genetically distinct red-flowering forms, one of which is dominant to pink and the other recessive. The inheritance of aleurone color in maize provides other parallel cases, for there is a white form dominant to purple and at least three recessive white forms are known which differ in a single gene from purple. Similarly in fowls reports show that the white of the White Leghorn is dominant to colored plumage while that of some other breeds, such as the Silky, White Plymouth Rock and the White Wyandotte, is recessive. In cattle a dominant white has long been known, and recently DETLEFSEN (1920) has described a recessive albino condition. In this last case the two whites are distinguishable, so that the parallelism is not as close as in the other cases. Many other instances of the same kind could be cited, but it is our purpose merely to point out a few parallel cases and not to catalogue all of them.

It is not always a simple matter to determine whether or not two genetically distinct characters, which are nearly, if not quite, identical phenotypically, may not possess some distinguishing morphological features. In the case of the red-flowering tobaccos, the authors comment at some length upon this difficulty. In describing and contrasting the various members of the "jaunty genus of mutants" in Drosophila, it is possible to enter into minute details with confidence, because it is practically certain that the residual genotypes in the various forms are identical, so that any differences among them are in all probability the effects of the mutant genes themselves. It is otherwise in the tobaccos, where such a detailed description of the difference in expression of the characters would be largely meaningless on account of the impossibility of determining without comprehensive tests whether the differences are dependent upon the existence of diverse residual genotypes or upon the effects of the particular genes under consideration. In such cases it is practically necessary to synthesize lines having the same residual genotypes before accurate conclusions can be drawn regarding the significance of slight character differ-

ences existing among the genotypically distinct members of a given phenotype. Here again we cannot refrain from calling attention to the distinct superiority of Drosophila for genetic investigations.

For those of us who are working largely with long-established cultivated forms, it is interesting to speculate upon the theoretical difficulties which may beset the investigator in the analysis of genetic phenomena in a given species. Thus if we had given us an original population consisting of jaunty, curled and ski, what a tangled skein we would have had to unsnarl! And yet we have no reason to believe that such complexities do not occur in our polymorphic species, whether wild or domesticated, and that they are not the cause of the apparent discrepancies which are met with in the literature of plant and animal genetics.

SUMMARY

1. Ski, a new wing character in *Drosophila melanogaster*, is shown to be genetically distinct from jaunty-X, jaunty, and curled, previously discovered mutant characters which it resembles phenotypically.

2. Ski is a bigenic character resulting from a change in two genes of the wild-type fly; one, S_{iii}, a second-chromosome dominant, and the other, s_{iii}, a third-chromosome recessive.

3. Neither S_{iii} nor s_{iii} produces any somatic change except when acting with the other.

4. The locus of S_{iii}, based on counts of 4845 flies in complementary back-crosses with *S* and *b* as reference points, is placed at II-30.8.

5. The locus of s_{iii}, based on counts of 15,621 flies in complementary back-crosses with *D* and s_s as reference points, is placed at III-43.4.

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